THE PREFERRED DECOMPOSITION LEVEL OF PECAN WOOD FOR AURICULARIA AURICULA-JUDAE MUSHROOM GROWTH

A Thesis

by

PEIYAO ZHANG

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Gerald L. Riskowski
Committee Members,	Ronald E. Lacey
	Zivko Nikolov
	Genhua Niu
Head of Department,	Stephen W. Searcy

May 2019

Major Subject: Biological and Agricultural Engineering

ABSTRACT

As mushrooms always grow on wood which is already decomposed, fresh wood is not suitable for fungus to live. The object of this research was to find the preferred decay level of Pecan logs for Auricularia auricular-judae (wood ear mushroom) growth. Both the log cultivation and bag cultivation methods were used to grow wood ear mushrooms in this research. These mushrooms were cultivated in an environmental growth chamber, which could control the environmental conditions (temperature, light, moisture, etc.) for mushroom growth. Pecan wood was used to cultivate the mushrooms. Physical and chemical properties of the pecan logs were measured to find the relationships between these properties and decay levels of logs.

There was a negative relationship between densities and decay levels of logs. The old decayed logs had lower densities than new fresh logs. Densities could be used as a basic standard to represent the decay levels. When the densities of logs were less than 430 $kg \cdot m^{-3}$, the negative linear relationship was significant between log hardness and densities of logs. The relationship between C/N ratios and densities of logs was not significant, except for logs that already had fruiting residue present.

Based on the log cultivation of wood ear mushroom, the spawn colonized, and mycelium appeared on logs with densities from 200 – 600 $kg \cdot m^{-3}$. There was no significant relationship between the densities and C/N ratio of these logs.

From the bag cultivation of wood ear mushroom, mushroom spawn colonized, and mycelium appeared in every bag. However, the growth conditions were different for each bag based on substrates. Growth bags with substrates made from logs with densities ranging 200 – 500 $kg \cdot m^{-3}$ had better mycelium growth then high-density ranges.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Riskowski, and my committee members, Dr. Lacey, Dr. Niu, and Dr. Nikolov, for their guidance and support throughout the course of this research.

I am really appreciated to my friends Yang and EJ for their help during construction of growth chamber. And Dr. Capareda, for using the analyzer and grinder in his lab.

Thanks also go to my colleagues and friends in the department faculty and staff for making my time at Texas A&M University a great experience.

Finally, thanks to my family for their encouragement and support during my graduate research.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supported by a thesis committee consisting of advisor Professor Gerald L. Riskowski of the Department Biological and Agriculture Engineering and Professor Ronald E. Lacey of the Department of Biological and Agriculture Engineering and Professor Zivko Nikolov of the Department of Biological and Agriculture Engineering and Professor Genhua Niu of the Department of Horticultural Sciences.

The data analyzed for chemical properties of the logs was provided by Professor Sergio Capareda of the Department of Biological and Agriculture Engineering.

All other work conducted for the thesis was completed by the student independently.

Funding Sources

There are no outside funding contributions to acknowledge related to the research and compilation of this document.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION

I.1 Literature Review

I.1.1 Background Introduction

Auricularia auricular-judae, also called wood ear mushrooms, is jelly fungi and basidiomycetes that grow in the tropics and subtropics. There are more than 20 species reported worldwide. It is widely distributed and regarded as a healthy food throughout the world, especially in Asia. China and Japan have been two of the biggest producers and consumers of wood ear mushrooms, and they also export mushrooms to many other countries. Wood ear mushrooms have become more and more popular around the world for its good flavor and simple cooking method.

I.1.2 Nutrition and Medical Function of Mushrooms

Wood ear mushrooms are regarded as a nutritional and healthy food. Like other edible fungi, it has a high portion of proteins, essential amino acids, unsaturated fatty acids, vitamins, macro, micro elements, polysaccharides and melanin (Kadnikova, et al., 2015). It is valued as a dietary product due to its low calories and absence of cholesterol. Hence, wood ear mushroom is very healthy and nutritious as diet food.

In the last few years, wood ear mushrooms have also been found to have medicinal values for anti-oxidative properties on aging mice (Zhang , et al., 2010). Oxidation is essential to many organisms to produce energy to fuel biological processes.

Nowadays, people are more concerned about their health and they purchase various products that may protect the human body from various types of oxidative damage that are linked to diabetes, cancer, and cardiovascular disease. Antioxidants are substances that can delay or prevent oxidation generally by scavenging free radicals. There are synthetic compounds that are strong radical scavengers. Research on natural antioxidants, with low cytotoxicity from plants, has become an important branch of biomedicine. In the traditional Chinese medicines, wood ear mushrooms have contributed to tonic and medical values. Recently, Chinese researchers and some Western researchers have become interested in finding new functional compounds in mushrooms. Some bioactive polysaccharides have been demonstrated to play an important role as a dietary free radical scavenger in the prevention of oxidative damage in living organisms.

I.1.3 Wood Ear Mushroom Life Cycle and Commercial Cultivation

1) Life cycle of wood ear mushroom

The wood ear mushroom exhibits bipolar heterothallism which means reproduction is via spores. The mushrooms multiply by producing millions and millions of spores. And when a spore settles in a suitable environment, it can germinate and branch to form a mycelium. When two sexually compatible mycelia meet, they may fuse to form a secondary mycelium (mature mycelium), which is capable of forming fruiting bodies. The fruiting body is produced by the mature mycelium and it finally grows to be the mushroom. In nature, the mushroom (bigger than 2 mm) is the most striking part of

the organism, but in fact it is just the fruiting body and the major part of the living organism is found under the ground or inside the wood.

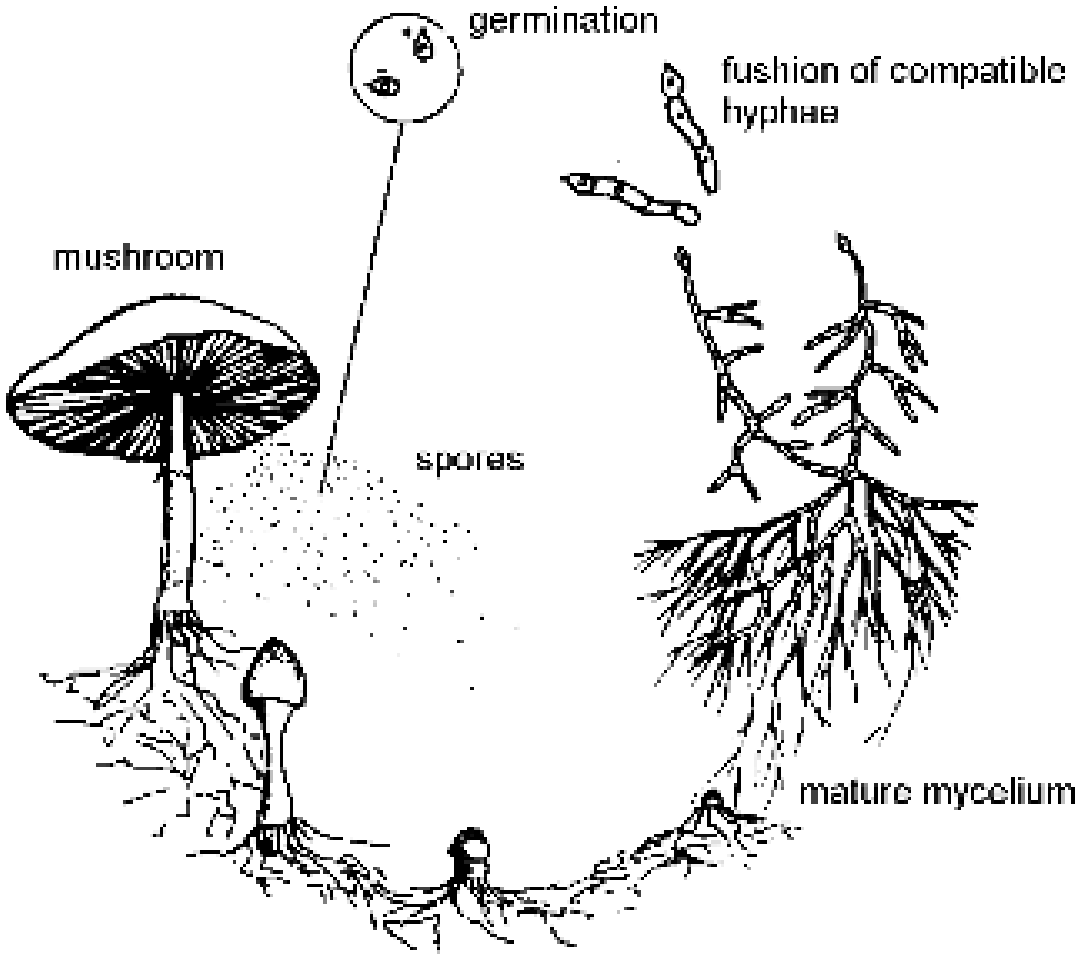


Figure I-1. Life cycle of mushrooms in nature, *adapted from Small-scale mushroom cultivation*, by Peter Oei, 2005

2) Edible mushroom cultivation

In the practice of edible mushroom cultivation, there is no use of spores. Because of their small size, they are difficult to handle. Moreover, it takes some time for spores to germinate. Some pre-grown mycelium of the mushroom is inoculated on a

sterile substrate. This material is referred to as spawn. These spawns can be used to grow mushrooms efficiently. Hence, the overall growth of the mushroom has been divided into 3 stages: 1) Inoculation of spawns. 2） Mycelium growth. 3) Development of fruiting body.

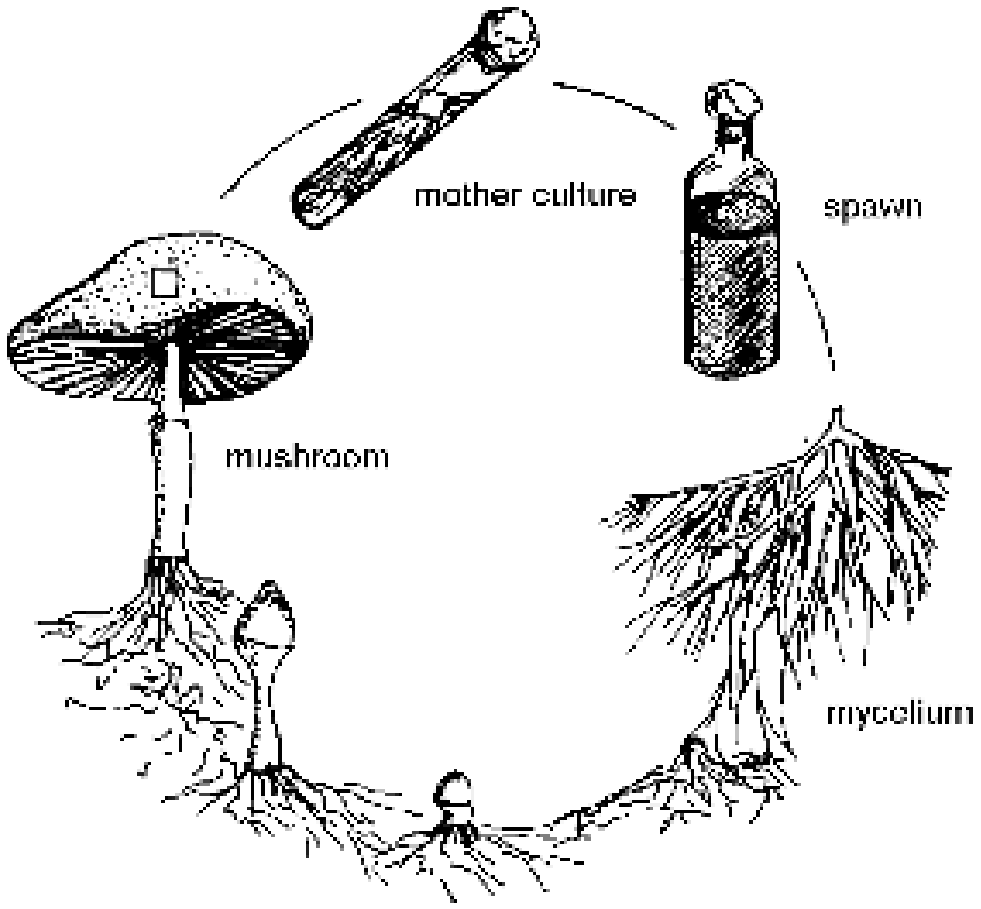


Figure I-2. Life cycle of mushrooms from spawn to mushrooms. Tissue culture are isolated from mushroom and propagated in substrate, *adapted from Small-scale mushroom cultivation*, by Peter Oei, 2005

I.2 Over-all Research Narrative

I.2.1 Main objective and hypothesis

Since Auricularia auricular-judae mushrooms grow on decayed wood rather than fresh wood, it is hypothesized that there is a specific optimal decay level of Pecan logs for Auricularia mushroom growth.

The main objective is finding the preferred decay level of Pecan logs for Auricularia auricular-judae mushroom growth. To determine the decay level and describe the relationship between log decay level and mushroom growth, the physical and chemical properties of logs were measured. The relationship between these properties and decay level were determined.

Hence, the objective is to determine the preferred decomposition level of pecan logs for wood ear mushroom growth. And wood ear mushroom growers and sellers will be able to measure pecan log decay levels and can cultivate mushrooms on the logs with the right decay level.

CHAPTER II

THE RELATIONSHIPS BETWEEN LOG PROPERTIES AND DECAY LEVELS

II.1 Introductions

II.1.1 Traditional decay level measurement

To measure the decay levels of logs, the traditional visual method divides them into 5 classes based on the visual examination (Daniels, et al., 1997). Logs from cool mesothermal forests of southwestern coastal British Columbia were used to determine the log decay levels. Those logs were divided into 5 classes by visual standards: i, ii, iii, iv, v

i) Freshly fallen logs, with sound bark and wood, and with presence of twigs.

ii) Sound bark and wood mainly, branches are present, but twigs are absent.

iii) Logs maintain structural integrity, bark is detached or absent, wood is decayed but still structurally sound.

iv) Logs are oval, bark is absent, and wood is soft due to decay, branch stubs can be removed.

v) Logs lack structural integrity and are being incorporated into the forest floor. Log height over the forest floor > half log diameter.

However, the classic method is inherently subjective, raising questions about repeatability. Such questions are of particular concern in practice, the class definitions often fail to clarify how to assign logs that are decomposing in an unusual way (e.g.,

bark still attached but can be broken by kicking). Therefore, such measurements presumably remain quite variable and inaccurate.

II.1.2 Use dynamic penetrometer to measure log hardness during decomposition

In a research paper (Larjavaara, et al., 2010), the wood decay levels were defined by their densities and hardness. During log decay processes, densities and hardness varied over time. The hardness of logs with different densities were measured by a penetrometer. From the penetration method, the dynamic penetrometer showed a strong relationship between log densities and penetration depth. There was a linear relationship between them (Larjavaara, et al., 2010). The density had a negative relationship with penetration depth. The research provided a good method to determine the log decay levels and proved to have higher precision and efficiency. However, some variations still existed. Logs with similar densities varied significantly in penetration depth by a dynamic penetrometer. This was not surprising, as hollow logs can be covered in strong bark and solid heartwood can be surrounded by decayed, soft sapwood. But this dynamic penetration method was better and more precise than the traditional visual classification.

II.1.3 The changes of chemical properties of logs during decomposition

During the wood decomposition process, not only the physical properties were changed, but the chemical properties of wood also changed. Especially the carbon, nitrogen and C/N ratio were significantly changed (Hale, et al., 1998). In one research project by Brais (2013), the chemical transformations were measured during the log

decomposition process. In this paper, the logs were divided into 5 classes by the traditional method of using visual cues. Then they measured the log densities and chemical properties. All the wood samples were ground in a ball mill to pass a 0.5 mm mesh screen before conducting the chemical analyses. Total C and N concentrations were determined by dry combustion using a CNS 2000 analyzer (LECO Corporation, St. Joseph, Michigan).

Logs from common deciduous and coniferous boreal species were studied, including white birch, trembling aspen, balsam fir, jack pine, white spruce, and black spruce. The study was conducted in the Lake Duparquet Research and Teaching Forest (48°86=N to 48°32=N, 79°19=W to 79°30=W), which is located in the Abitibi region of northern Quebec, 45 km north-west of the city of Rouyn-Noranda. Densities of logs ranged from 79 to 469 $kg \cdot m^{-3}$. Despite a general decrease in wood density with log decay classes, densities of adjacent decay classes overlapped for all species, and large variations in wood density were observed within each decay class.

The results showed that C concentrations increased significantly with decreasing density, from 492 to 502 $\mathrm{mg \cdot g^{-1}}$ in deciduous species, from 515 to 533 $\mathrm{mg \cdot g^{-1}}$ in jack pine, and from 503 to 519 $\mathrm{mg \cdot g^{-1}}$ in spruce spp. And, from 501 to 512 $\mathrm{mg \cdot g^{-1}}$ in balsam fir. Nitrogen concentrations in logs also significantly increased with decreasing wood density, from 0.87 to 6.32 $\mathrm{mg \cdot g^{-1}}$ in deciduous species, 0.73 to 1.93 $\mathrm{mg \cdot g^{-1}}$ in balsam fir, 0.49 to 2.07 $\mathrm{mg \cdot g^{-1}}$ in jack pine, and 0.42 to 1.98 $\mathrm{mg \cdot g^{-1}}$ in spruce spp. But nitrogen concentrations remained higher in deciduous logs than in coniferous logs throughout decomposition, and higher in white birch than in trembling aspen. The C/N

ratios decreased significantly with decreasing wood density, from 508 to 79 for white birch, 562 to 100 in aspen, 686 to 260 in balsam fir, and 1051 to 257 in jack pine. Only spruce spp increased from 1197 to 1565 at early decay stage, then decreased to 262 at late decay stage. The C/N ratios of deciduous logs remained lower than those of coniferous logs as density decreased and were lower for white birch than for trembling aspen. Overall, the chemical properties were changed during the log decomposition process. The relationship between C/N ratios and decay levels were obvious, but the effect of tree species and patterns existed.

Table II-1. The changed densities, carbon and nitrogen concentration with different decay classes. ***Adapted from Brais, 2013***

Species (logs)	Decay classes	Density ($kg \cdot m^{-3}$)	Carbon ($mg \cdot g^{-1}$)	Nitrogen ($mg \cdot g^{-1}$)	C/N
White birch	i & ii	351±120	498±2	0.95±0.58	580.16
	iii	303±64	505±10	1.12±0.35	450.89
	iv	293±120	508±11	1.73±0.64	293.64
	v	171±83	501±11	6.32±3.18	79.27
Trembling aspen	i & ii	326±47	489±5	0.87±0.64	562.07
	iii	244±39	499±4	0.71±0.23	702.82
	iv	211±48	493±5	1.36±0.56	362.50
	v	157±38	498±12	4.95±2.12	100.61
Balsam fir	i & ii	316±31	501±8	0.73±0.44	686.30
	iii	223±28	511±9	0.93±0.24	549.46
	iv	207±30	512±9	1.18±0.36	433.90
	v	197±41	502±8	1.93±0.86	260.10
Jack pine	i & ii	339±44	515±7	0.49±0.15	1051.02
	iii	289±45	532±11	0.65±0.26	818.46
	iv	275±40	530±11	0.68±0.19	779.41
	v	207±34	533±18	2.07±0.86	257.49
Spruce spp.	i & ii	346±46	503±2	0.42±0.10	1197.62
	iii	283±74	501±5	0.31±0.11	1565.63
	iv	213±6	527±2	1.50±1.37	351.33
	v	199±73	519±21	1.98±0.49	262.12

II.2 Objectives and hypothesis

II.2.1 Relationships between log physical properties and decay levels

The objective was to measure the changes of physical properties of logs during log decomposition process, and to find the relationships between these physical properties and decay levels.

Densities changed during the log decay process. It was hypothesized that there was a relationship between density and decay levels, so densities can be used to represent the log decay levels. Hardness also varied during log decomposition process. Therefore, it was hypothesized that a relationship existed between log hardness and decay level.

II.2.2 The changes of chemical properties of logs during decay

The objective was to find the relationship between log chemical properties and decay levels. The carbon, nitrogen and C/N ratio of the logs were measured to determine the association between these chemical properties and log decay levels. Differences of carbon, nitrogen and C/N ratio of logs with different densities were analyzed. The relationships between them and log densities were determined.

It was hypothesized that the relationship between C/N ratio and decay levels can be used to represent log decay levels.

II.3 Methodology

II.3.1 Logs pre-treatment

In this research, the logs with different decay levels were selected from several different Pecan trees in central Texas. First, all logs were cut into 1-foot-long lengths with different diameters. After that, they were put in a small lab room at 23℃ and 40% relative humidity. The logs were conditioned for at least one week before the measurement of all physical properties could start.

II.3.2 Physical properties measurement

To measure the log moisture content, the MD918 Intelligent Moisture Meter was used. The moisture meter can directly read the moisture contents by contacting object surface within a range from 4% to 80% by mass basis. The maximum error was $(RH \cdot 1\% + 0.5)$ and resolution was 0.5%. It can be calibrated easily to adjust to the external environment. It had several measurement classes with different upper limits of moisture content. The upper limit also could be changed and calibration status was set to adjust to the external environment before measurement. It had several measurement classes with different upper limits of moisture content.

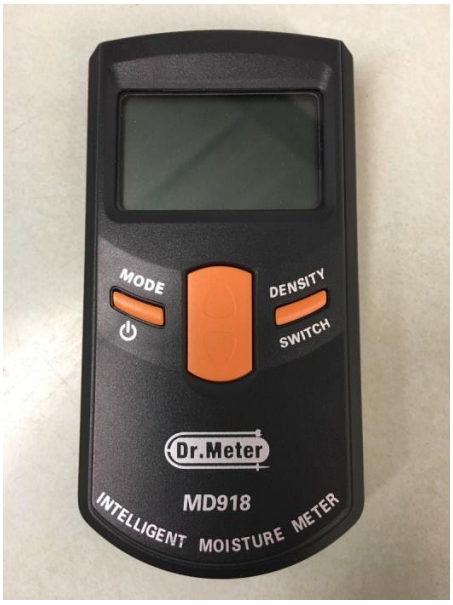


Figure II-1. MD918 moisture meter

The masses of the logs were measured by a Mettler Toledo PB3002S accurate balance. The maximum capacity of the balance is 3100 g with increments of 0.01g. The volumes were determined by drainage method in a cuboid shaped box. Water was added into the box, and the line of water level was marked. Then each log, wrapped with a thin plastic bag, was put into the box and pushed under the water level. Mark the water level again. The volume of log equaled the volume of water between the two lines. The dry masses of logs were calculated by total masses and deducting the moisture content in the logs. Hence, densities were calculated by dividing the dry mass by volume of logs. To find the relationship between physical properties and decay levels of logs, 104 logs were measured with densities from over 200 $kg \cdot m^{-3}$ to 800 $kg \cdot m^{-3}$. These logs were divided into 6 categories by density for every 100 $kg \cdot m^{-3}$ from 200 $kg \cdot m^{-3}$ to 800

$kg \cdot m^{-3}$. These density categories were labeled by numbers from 1 to 6; 1 being the least dense.

Figure II-2. Mettler Toledo PB3002S accurate balance

In here, the hardness of logs was represented as penetration depth. It was measured by a dynamic penetrometer (Figure II-3) (Larjavaara, et al., 2010). The penetrometer was composed of 5 parts. The moving weight was 1 kg, dropping distance of the moving weight was 250 mm, and the diameter of the pin was 5 mm. When measuring the log penetration, the moving weight was dropped vertically from 250 mm height for 20 times. After 20 hits, the pin was marked at the location parallel to the log

surface. The distance from the pin tip to the marked place was penetration depth. If the penetration depth reached 55 mm in hits less than 20 times, then the hit numbers were also recorded. The logarithm of the penetration in millimeters per hit was used in the analyses.

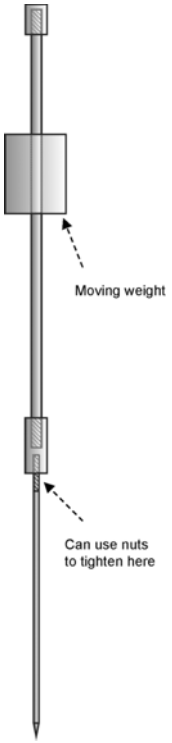


Figure II-3. The dynamic penetrometer (Larjavaara, 2010)

II.3.3 Chemical properties measurement

In previous research, the carbon and nitrogen of boreal hardwood trees in Canada had been measured and the relationship between C/N ratio and log density were determined (Brais, et al., 2013). In this research, the carbon and nitrogen contents and C/N ratio of Pecan trees were also measured. These C/N ratios were compared to

previous data of boreal trees to find if the species and environments were the significant factor in the relationship.

Three logs were selected from each of the density categories. The logs with bigger diameter were preferred to choose for chemical properties measurement and mushroom growth. As mentioned earlier, the log densities varied from 200 to 800 $kg \cdot m^{-3}$, and the logs were divided into 6 categories for every 100 $kg \cdot m^{-3}$ interval. Hence, there were 18 logs that were measured and used to cultivate mushrooms. The C and N values of these logs were measured by Vario MICRO cube elemental analyzer (Elementar, Inc).

Figure II-4. The 18 logs selected for C/N ratio measurement

Log samples were collected by using an electric drill to drill logs on the left, right and middle parts to 3-5 cm depth. Each part was done in triplicate. Samples were taken from these 9 locations on every log. So 9 samples of each log and 3 logs of each density category were prepared for carbon and nitrogen contents test. For samples on the same log, the samples were mixed together. Then they were ground to 40 mesh (0.420 mm) and dried in an oven. Samples were dried at 80℃ for 4 h. After that, they were weighed to 2 mg samples for combustion in the Vario MICRO cube elemental analyzer.

Figure II-5. Vario MICRO cube elemental analyzer, *adapted from Elementar, Inc*

The samples were sealed with aluminum foil and put in the tubes of the analyzer to analyze the chemical compositions. The wood decomposition process was really complicated. The samples were collected from decay wood. To determine what exact chemical compositions of these samples were fairly difficult. But it was white rot type decomposition for edible mushroom growth. During this process, cellulose and lignin decompositions were two dominant decay processes. The cellulose was lysed by cellulase to organic compounds glucose, cellobionic acid and gluconic acid as early

products (Kirk, et al., 1984). Later, the soluble and low weight products which would provide nutrition for fungus growth were produced. And the initial product of lignin lysed by ligninase was vanillic acid (Kirk, et al., 1984). Late products were some soluble and low weight compounds for fungus growth. However, the samples were combusted in the analyzer columns. The whole chemical compositions were under fully combustions to convert these compounds to the final products of carbon dioxide, nitrogen dioxide, sulfured dioxide and water. These final products of combustion were analyzed in the detection system which depended on the sample type and size. Therefore, the final chemical compositions (carbon, nitrogen, sulfur and hydrogen) were determined.

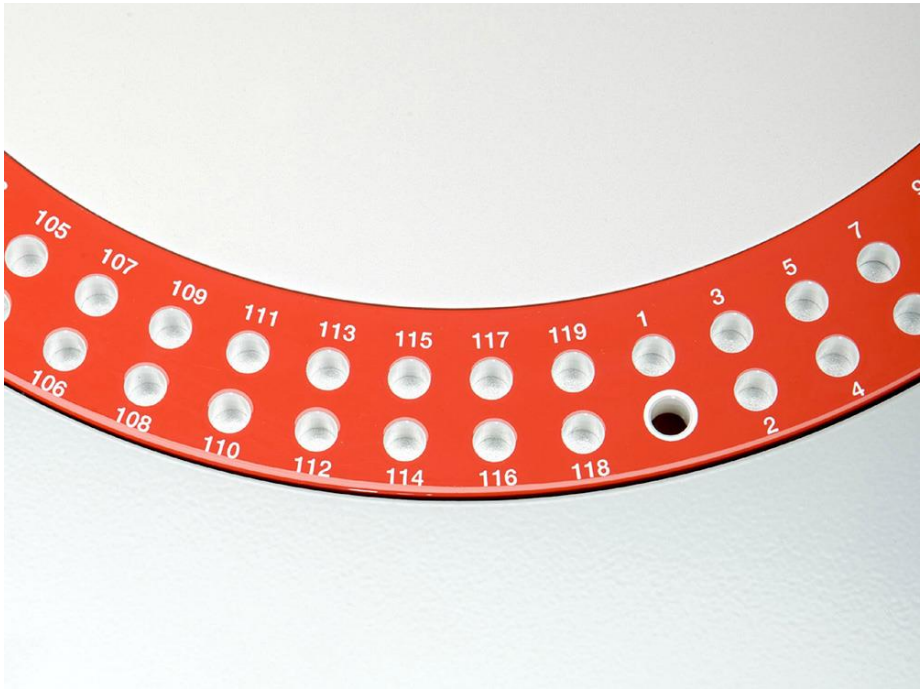


Figure II-6. The column tubes for element analysis, *Reprinted from Elementar, Inc*

Figure II-7 showed the analysis process for a single sample in the analyzer. In the micro cube, a multi-column technology was utilized to separate even larger sample size. The gaseous compounds were adsorbed at element specific columns and one after the other released by a temperature support desorption. All elements were analyzed simultaneously from the one sample.

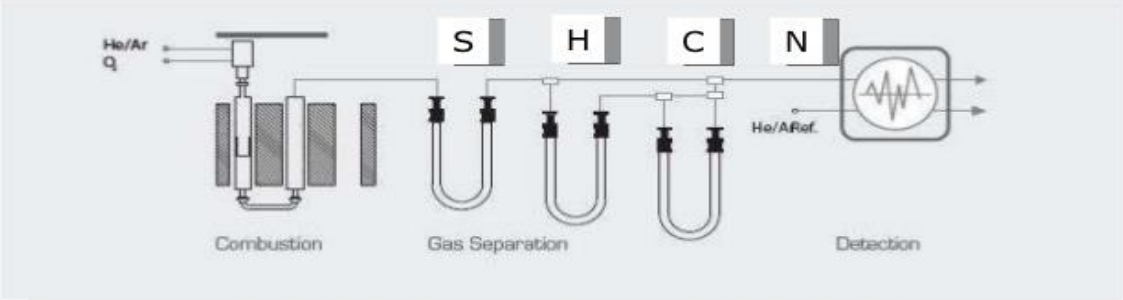


Figure II-7. The analysis principle of elemental analyzer. ***Reprinted from Slideshare, 2011***

II.4 Results and Discussions

II.4.1 Physical properties of all 104 logs

The density and logarithm of penetration depth of all 104 logs were measured. All logs were divided into 6 categories by density for every 100 $kg \cdot m^{-3}$ interval from 200 to 800 $kg \cdot m^{-3}$, category 1 represented the lowest density. The average and standard deviation of density and logarithm of penetration depth were measured, Table II-2.

Table II-2. Data of physical properties of all 104 logs.

Category	Density ($kg \cdot m^{-3}$)	Density average ($kg \cdot m^{-3}$)	Density standard deviation	Logarithm of penetration average (mm)	Logarithm of penetration standard deviation (mm)
1	200-300	273.87	18.70	0.38	0.11
2	301-400	357.06	25.62	0.44	0.35
3	401-500	462.34	24.46	0.20	0.12
4	501-600	549.19	27.04	0.14	0.02
5	601-700	648.63	26.55	0.14	0.02
6	701-800	725.22	18.09	0.13	0.01

II.4.2 Decay levels of the 104 logs

In here, the decay levels of the 104 logs were determined by the traditional visual method. Logs with high densities had low decay levels and low densities logs had high decay levels, Table II- 3. The number of logs at each density category had also counted. Most logs were at density range from 300 to 700 $kg \cdot m^{-3}$.

Table II-3. Wood decay levels by visual method versus densities of 104 logs

Decay levels / Density range ($kg \cdot m^{-3}$)	i	ii	iii	iv	v	No. of Logs by density category
200 - 300 (1)	0	0	0	1	5	6
301 - 400 (2)	0	0	3	9	2	14
401 - 500 (3)	0	2	10	8	1	21
501 - 600 (4)	0	2	12	3	0	17
601 - 700 (5)	6	23	8	0	0	37
701 - 800 (6)	6	3	0	0	0	9
No. of Logs by decay levels	12	30	33	21	8	104

Figure II-8 showed the normal distribution fit of densities of all logs. The fit was smooth and normal distribution was satisfied.

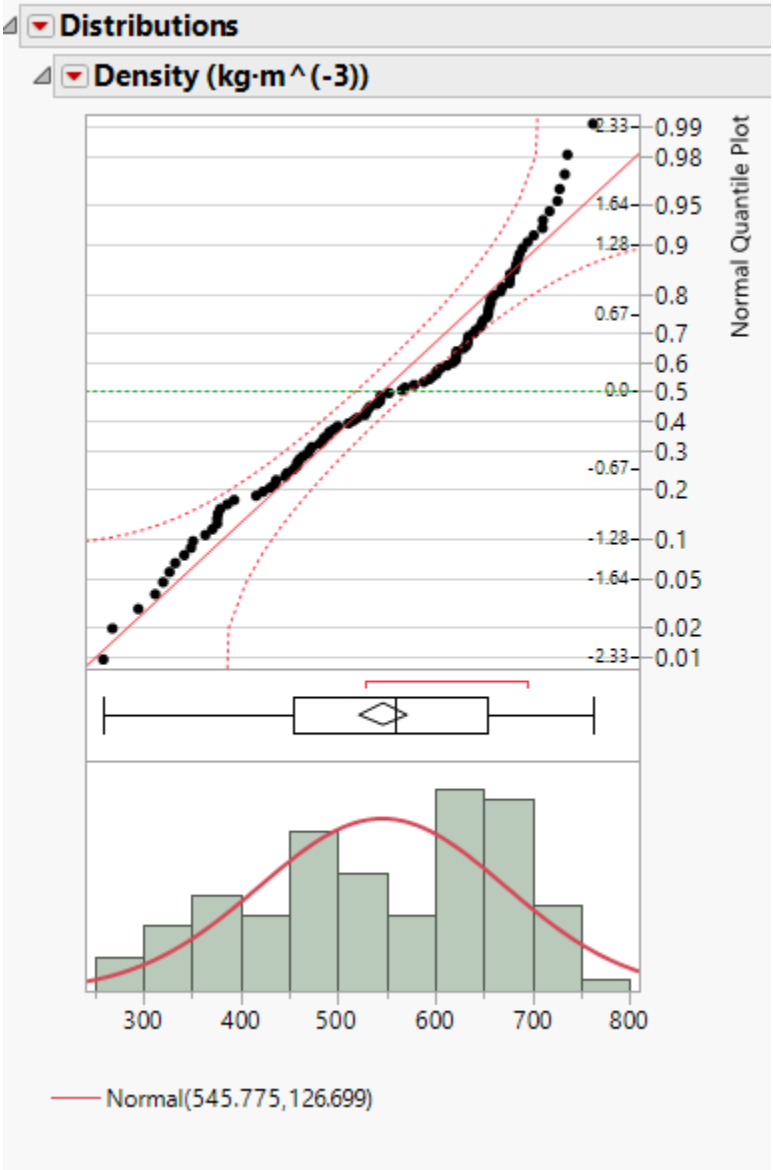


Figure II-8. Distribution of densities of 104 logs $(kg \cdot m^{-3})$

However, the normal distribution of penetration depth was not satisfied based on Figure II-9. It showed most values of logarithm were less than 0.2 mm.

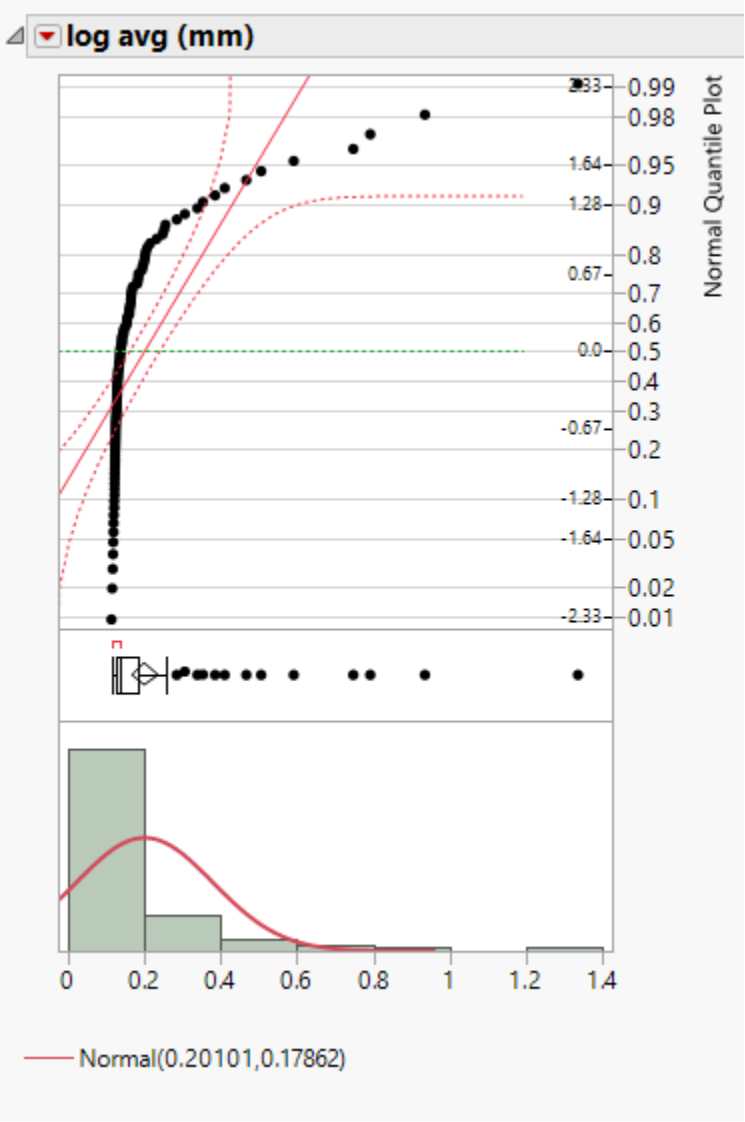


Figure II-9. Distribution of logarithms of penetration depth (*mm*)

The average and standard deviation of moisture content of all logs were also calculated, Table II-4. The average moisture content of all logs was 7.93%, and standard deviation was 6.72.

Table II-4. The mean and standard deviation of moisture content (%) of 104 logs

Mean	7.93
Standard Deviation	6.72
Standard Error Mean	0.66
Upper 95% mean	9.24
Lower 95% mean	6.63
No. of samples	104

The distribution of log moisture content was not a normal distribution based on Figure II-10, and the fit was not very smooth.

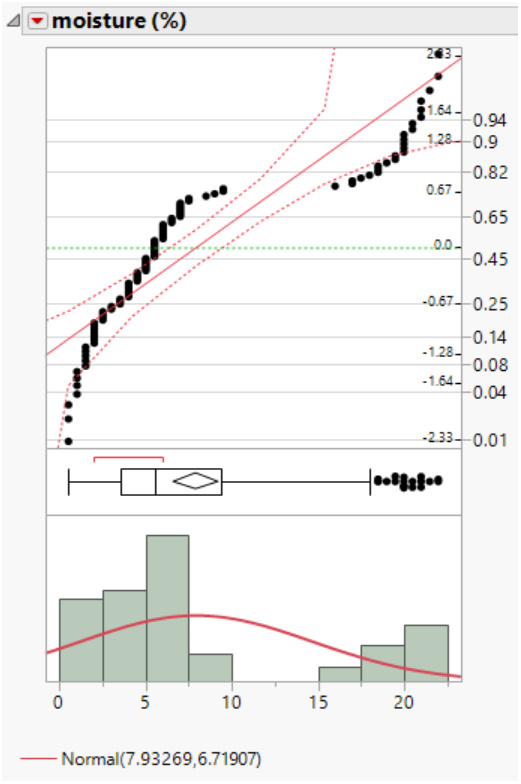


Figure II-10. The distribution of moisture content (%) **of 104 logs**

II.4.3 The physical and chemical properties of the selected 18 logs

Table II-5 showed the data of densities, logarithm of penetration depth and C/N ratio of the 18 logs used for mushroom growth.

Table II-5. The overall physical and chemical properties of the 18 logs used for mushroom growth

Category	Density Range ($kg \cdot m^{-3}$)	Density Avg. ($kg \cdot m^{-3}$)	Density SD	Penetration Avg. (mm)	Penetration SD	C/N Avg.	C/N SD
1	200-300	273.87	18.70	0.38	0.11	93.57	68.42
2	301-400	363.86	12.83	0.25	0.06	91.85	56.21
3	401-500	474.18	23.13	0.15	0.02	112.38	13.14
4	501-600	537.58	7.44	0.14	0.01	107.52	20.43
5	601-700	647.42	37.67	0.14	0.02	107.13	20.09
6	701-800	733.72	26.40	0.13	0.01	60.44	16.27

The average diameters of the 18 logs were also measured. From Figure II-11, it was a normal distribution for the diameters of these logs.

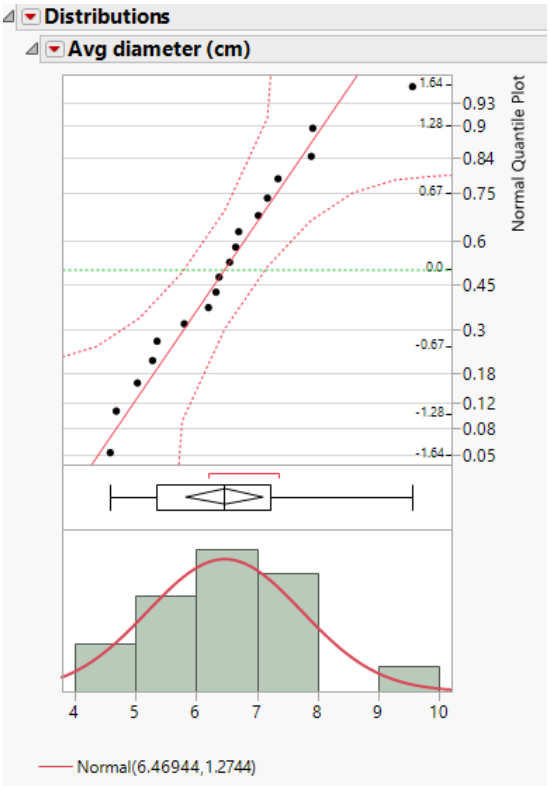


Figure II-11. The distribution of diameters (cm) of the 18 logs used for mushroom growth

The mean value of log diameters was 6.47 cm and standard deviation was 1.27 for the 18 logs used for mushroom growth, Table II-6.

Table II-6. The mean and standard deviation of diameters (cm) of the 18 logs used for mushroom growth

Mean	6.47
Standard Deviation	1.27
Standard Error Mean	0.30
Upper 95% mean	7.10
Lower 95% mean	5.84
No. of samples	18

II.4.4 Relationships between log hardness and densities

The penetration depths had been measured 3 times for each log; on the left, middle and right part. Figure II-12 showed the fit of log density versus logarithm of penetration depth of all 104 logs. When densities were larger than 430 $kg \cdot m^{-3}$, there were no significant difference of logarithm of penetration depths when densities changed. However, there was a negative relationship between log density and logarithm values when density was below 430 $kg \cdot m^{-3}$. For all charts with densities, densities were on dry weight basis. The orange triangle marks were the 18 logs selected for chemical properties measurement and mushroom growth.

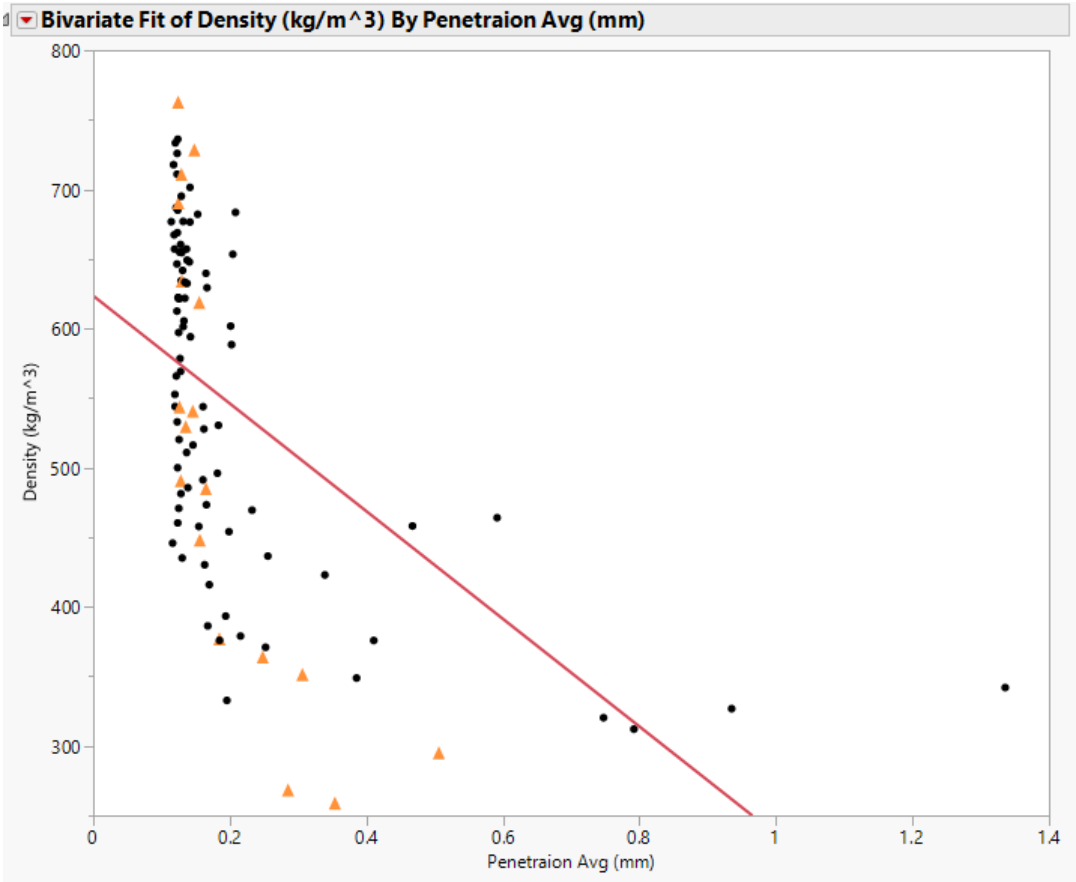


Figure II-12. Log density ($kg \cdot m^{-3}$) versus logarithm of penetration depths (mm) of 104 logs; Orange triangle were logs selected for chemical properties measurement and mushroom growth

Since the P-value was less than 0.0001, the linear model between density and logarithm was significant, Table II-7.

Table II-7. ANOVA analysis of log density ($kg \cdot m^{-3}$) (d.w) versus logarithm of penetration depth average (mm)

Summary of fit	RSquare		0.30
	Rsquare Adj		0.29
	Root Mean Square Error		106.70
	Mean of Response		545.78
	Observations (or Sum Wgts)		104
Analysis of Variance	F Ratio		43.22
	Prob > F		< 0.0001
Parameter Estimates	Intercepts	Estimate	623.56
		t Ratio	39.48
		Prob > |t|	< 0.0001
	Penetration Avg	Estimate	-386.97
		t Ratio	-6.57
		Prob > |t|	< 0.0001

Figure II-13 gave the relationship between density and logarithm of penetration of two separate fits which were closer to realistic conditions. It showed that for log densities from 230 to 430 $kg \cdot m^{-3}$, an obvious negative relationship existed. When densities varied from 430 to 800 $kg \cdot m^{-3}$, the first curve showed the penetration depths changed very little. Hence, the second curve indicated that a strong negative relationship existed between density and the logarithm of penetration depth.

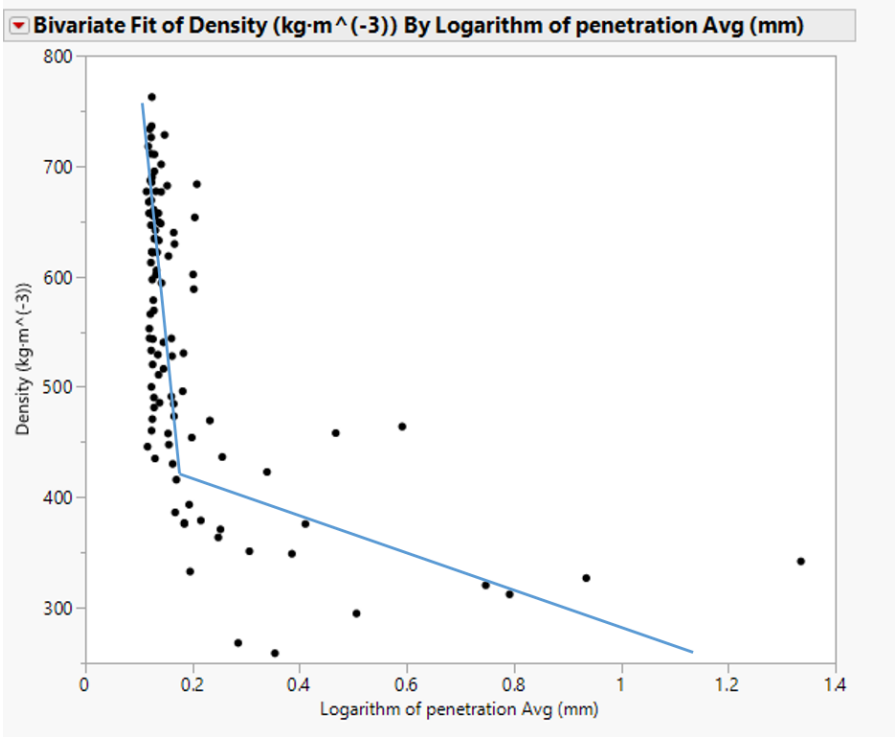


Figure II-13. Density $(kg \cdot m^{-3})$ versus average of logarithm of penetration depth (mm) per hit numbers of 104 logs

For the 18 logs selected for the mushroom growth tests from all 104 logs, the relationship between densities and logarithm of penetration was also determined. Figure II-14 showed the relationship between densities and logarithm of penetration of these selected 18 logs, and it appeared that a linear negative relationship existed with an R squared value equal to 0.5768.

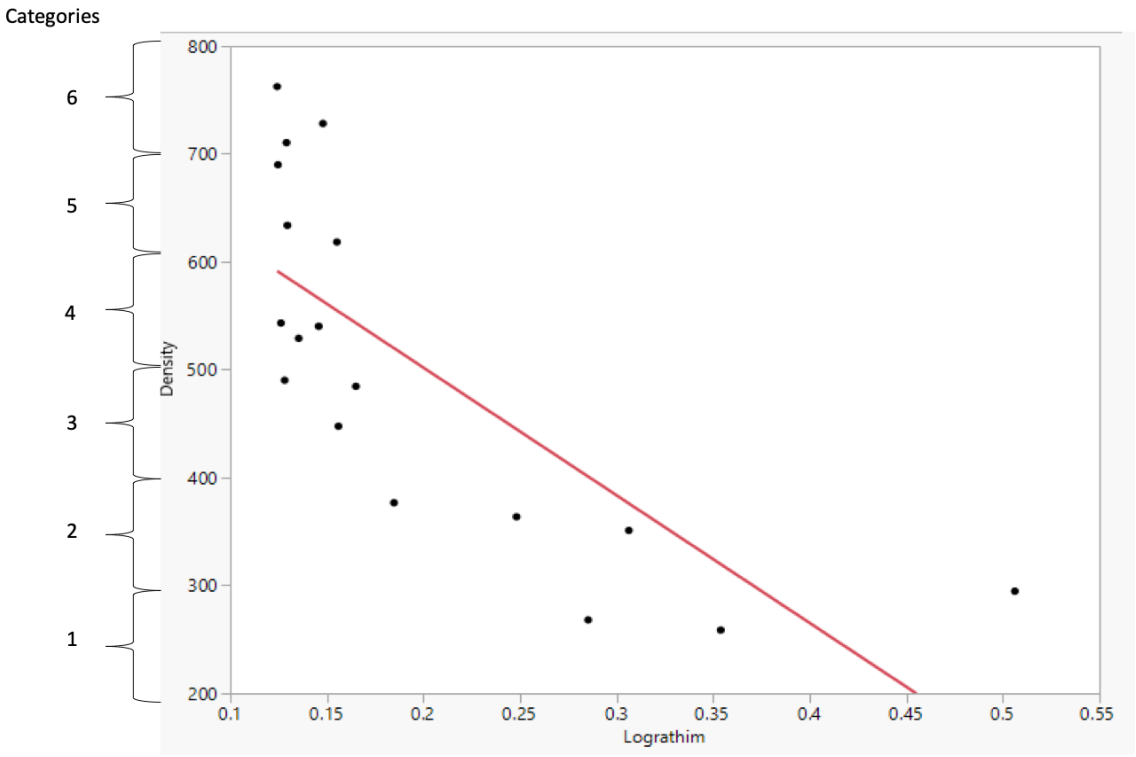


Figure II-14. Density $(kg \cdot m^{-3})$ versus logarithm of penetration depth (mm) per hit numbers of selected 18 logs

From Table II-8, the P-value was smaller than 0.05, so a linear relationship existed between log densities and the logarithm of penetration depths.

Table II-8. ANOVA analysis of log densities ($kg \cdot m^{-3}$) versus logarithm of penetration (mm)

Summary of fit	RSquare	0.58
	Rsquare Adj	0.55
	Root Mean Square Error	109.13
	Mean of Response	505.10
	Observations (or Sum Wgts)	18
Analysis of Variance	F Ratio	21.81
	Prob > F	0.0003

II.4.5 Variations of chemical properties during log decay process

The carbon and nitrogen contents were measured for the 18 logs before mushroom cultivation. Based on the statistical analysis, the relationship between chemical properties and log decay process was determined.

From Figure II-15, the linear relationship was not satisfied for carbon content versus log densities. There was a slight trend, however, the data had wide variations for most of the logs, so the carbon content decreased slightly when the densities increased. Hence, the carbon content increased slightly during log decay process.

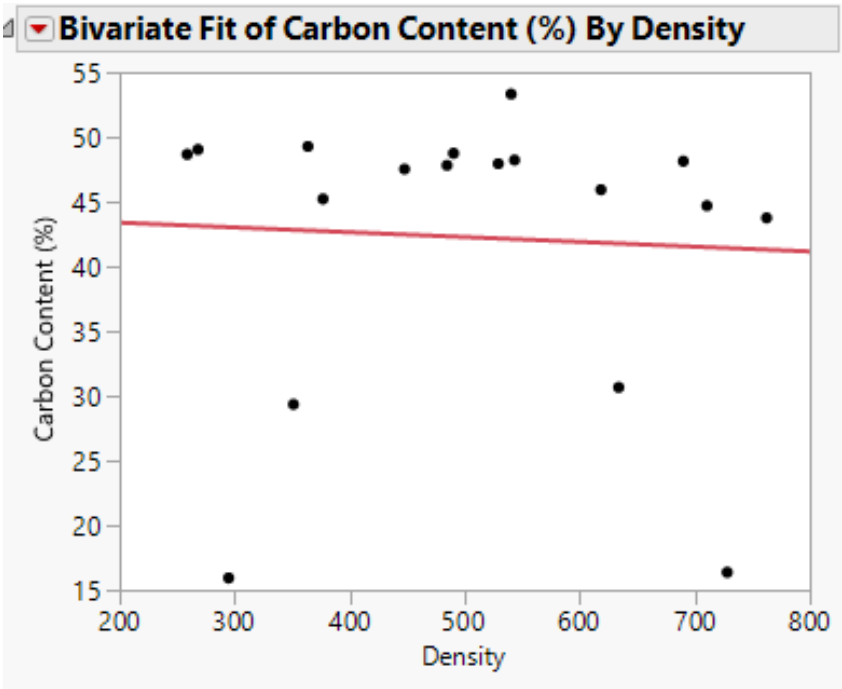


Figure II-15. The carbon content (%) versus density $(kg \cdot m^{-3})$ of the 18 logs used for mushroom growth

The R squared was 0.0028 and P-value was 0.8327, Table II-9. Hence, the linear relationship was not significant for carbon contents versus densities of these logs.

Table II-9. The ANOVA analysis of carbon content (%) versus density ($kg \cdot m^{-3}$) of the 18 logs used for mushroom growth

Summary of fit	RSquare		0.003
	Rsquare Adj		-0.06
	Root Mean Square Error		11.59
	Mean of Response		42.26
	Observations (or Sum Wgts)		18
Analysis of Variance	F Ratio		0.05
	Prob > F		0.83
Parameter Estimates	Intercepts	Estimate	44.13
		t Ratio	4.83
		Prob > |t|	0.0002
	Density	Estimate	-0.004
		t Ratio	-0.21
		Prob > |t|	0.83

From Figure II-16, there was no significant linear relationship between nitrogen content and density. There was a slight trend where the nitrogen increased slightly with increasing density for most logs. Therefore, the nitrogen content decreased slightly during log decomposition.

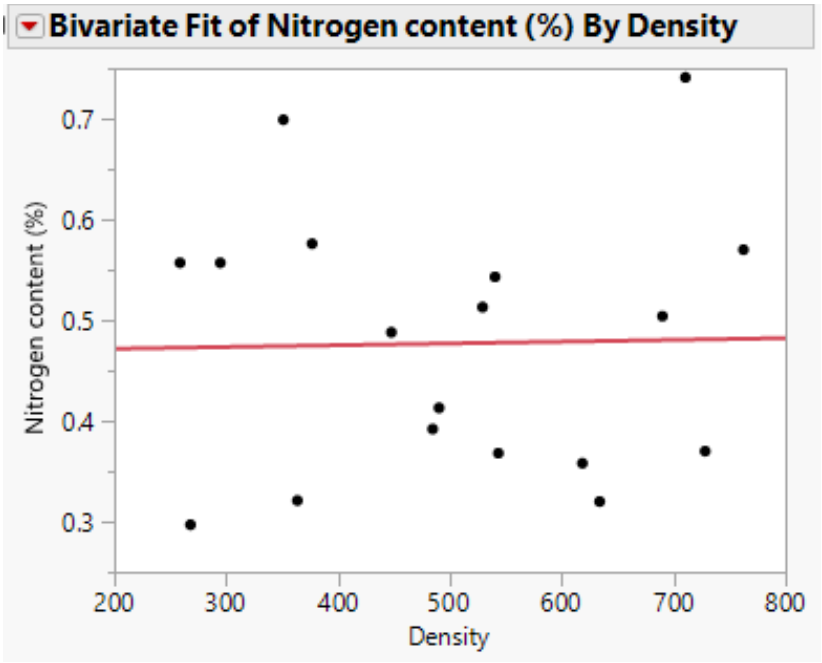


Figure II-16. The nitrogen content (%) versus density $(kg \cdot m^{-3})$ of the 18 logs used for mushroom growth

The R squared value was 0.0005 and P-value was 0.9303 in Table II-10. Therefore, there was no significant linear relationship for nitrogen content versus log density.

Table II-10. The ANOVA analysis of nitrogen content (%) versus density ($kg \cdot m^{-3}$) of the 18 logs used for mushroom growth

Summary of fit	RSquare		0.0005
	Rsquare Adj		-0.06
	Root Mean Square Error		0.13
	Mean of Response		0.48
	Observations (or Sum Wgts)		18
Analysis of Variance	F Ratio		0.008
	Prob > F		0.93
Parameter Estimates	Intercepts	Estimate	0.47
		t Ratio	4.43
		Prob > |t|	0.0004
	Density	Estimate	$1.77e^{-5}$
		t Ratio	0.09
		Prob > |t|	0.93

The C/N ratios of the 18 logs were also measured. Figure II-17 showed that there was no strong linear relationship between C/N ratio and densities. For C/N decreased slightly with increasing density, it indicated that C/N increased slightly during log decay process.

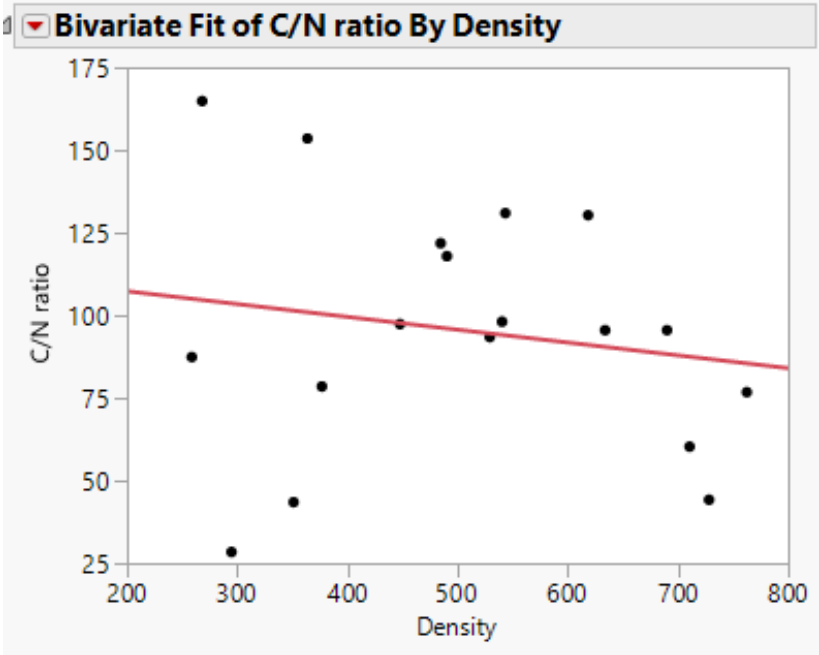


Figure II-17. C/N ratio versus density ($kg \cdot m^{-3}$) of 18 logs used for mushroom growth

From Table II-11, the R square was only 0.028 in the ANOVA analysis, and the P-value was 0.5 which was much larger than 0.05. Hence, the linear trend that C/N may decrease slightly with increasing log density was not significant.

Table II-11. The ANOVA analysis of C/N ratio versus density ($kg \cdot m^{-3}$) of 18 logs used for mushroom growth

Summary of fit	RSquare		0.03
	Rsquare Adj		-0.03
	Root Mean Square Error		37.88
	Mean of Response		95.48
	Observations (or Sum Wgts)		18
Analysis of Variance	F Ratio		0.47
	Prob > F		0.50
Parameter Estimates	Intercepts	Estimate	115.08
		t Ratio	3.85
		Prob > |t|	0.001
	Density	Estimate	-0.04
		t Ratio	-0.69
		Prob > |t|	0.50

Based on Figure II-18, the polynomial plot showed a better fit than the linear plots. However, the R square was still only 0.129 and the P-value was 0.35 and still larger than 0.05 in Table II-12. Therefore, the relationship was not significant.

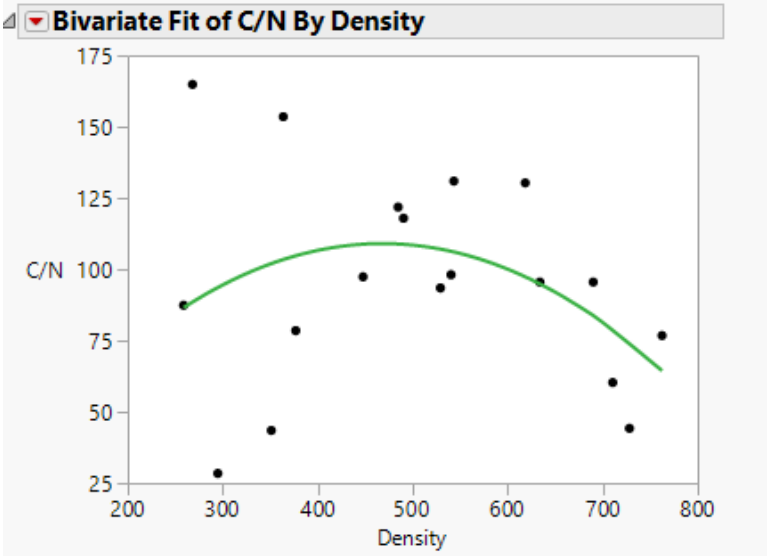


Figure II-18. C/N ratio versus density $(kg \cdot m^{-3})$ of polynomial plot for 18 logs used for mushroom growth

Table II-12. The ANOVA analysis of C/N ratio versus density ($kg \cdot m^{-3}$) of polynomial plot for 18 logs used for mushroom growth

Summary of fit	RSquare	0.13
	Rsquare Adj	0.01
	Root Mean Square Error	37.03
	Mean of Response	95.48
	Observations (or Sum Wgts)	18
Analysis of Variance	F Ratio	1.11
	Prob. > F	0.35

Figure II-19 gave the plot of average log densities versus C/N ratio based on density categories. The plot showed that a slight polynomial relationship existed between them. However, it could be estimated that when log density was around 500 $kg \cdot m^{-3}$, C/N ratio reached its maximum value from Figure II-19.

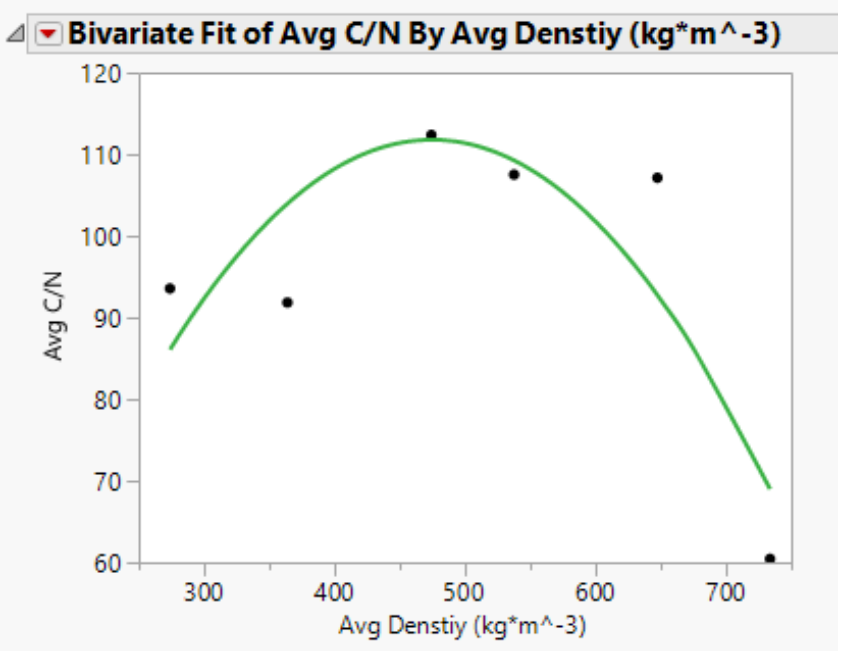


Figure II-19. The average C/N ratio versus averaged densities ($kg \cdot m^{-3}$) by density categories of 18 logs used for mushroom growth

From the Table II-13, the R square was 0.7316. And the P-value was 0.14 which was still larger than 0.05, so there was no significant polynomial association.

Table II-13. The ANOVA analysis of average C/N ratio versus log density of 18 logs used for mushroom growth

Summary of fit	RSquare	0.73
	Rsquare Adj	0.55
	Root Mean Square Error	12.73
	Mean of Response	95.48
	Observations (or Sum Wgts)	6
Analysis of Variance	F Ratio	4.09
	Prob > F	0.14

II.5 Conclusions

II.5.1 The relationship between log decay levels and physical properties

Based on the traditional visual methods of determining log decay levels, it is known that fresh logs usually have high densities and moisture contents. And, that old decayed logs always have lower densities and moisture contents. Hence, the densities can be used as a basic standard to represent the wood decay levels.

The hardness of logs was regarded as an important physical property for the logs. It was measured as penetration depth in this research. From the previous part, there was no significant relationship between log densities and penetration depths when the log densities were larger than 430 $kg \cdot m^{-3}$. However, for Pecan logs, the penetration depths significantly increased with decreasing log densities when the log densities were below 430 $kg \cdot m^{-3}$. Therefore, it could be concluded that the penetration depths increased during the log decomposition process.

II.5.2 The variation of chemical properties during the log decomposition process

As the enzymes activities decomposed logs, the carbon and nitrogen contents were changed. However, the variation in the carbon contents of the logs in this study did not show a significant linear relationship with log density. There was only a slight trend shown where carbon contents increased slightly with decreasing log densities, Figure II-15. So there was slight evidence that the carbon contents increased during pecan log decay process within the range of log densities tested in this study.

For the nitrogen contents, there was no significant linear relationship with pecan log densities. But there was a slight trend where the nitrogen contents decreased when log densities decreased, Figure II- 16. Hence, nitrogen contents might decrease slightly during the log decomposition process within the range of log densities tested in this study.

However, discussing the carbon or nitrogen contents separately wasn't as important as the C/N ratio. For all the logs, the linear relationship between C/N ratio and pecan log densities was not significant. There was a slight trend where the C/N ratio increased with decreasing log densities but there was wide variation in the data.

Previous research has shown that during the log decay process, the carbon contents increased significantly with decreasing densities. And nitrogen contents also increased significantly when densities decreased. However, the changing of nitrogen contents was more significant then carbon contents. Hence, the C/N ratio decreased with decreasing densities. It concluded that C/N ratio decreased during log decomposition process. The boreal hardwood trees were observed in Canada in previous research compared to Pecan tree in Texas in this research.

CHAPTER III

LOG CULTIVATION OF WOOD EAR MUSHROOMS

III.1 Introduction

III.1.1 Literature review

Log cultivation of mushrooms is fairly simple and believed to be the best method to save nutritional value inside the mushroom. Under the natural environment, the mushroom fungus usually prefers to infect woods which are already partially decomposed. During the decomposition process of logs, the physical and chemical properties of logs are changed by microbial activities. The nitrogen, carbon and mineral resources have significant effects on mushroom growth (Jonason, et al., 2001). The ligninase, cellulase and other enzymes make logs become ideal nutrition sources for fungi growth (Luo, 1993). However, how the enzymes changed the wood and what contents are changed by them are not usually of interest to mushroom growers. How to grow mushrooms efficiently and economically are the primary demands for them.

Pecan trees are common local hardwood trees in Central Texas and cultivating mushrooms on them is an economic and realistic way for local mushroom growers. Hence, finding the right decay level of logs for mushroom growth can save cultivation time, costs and improve mushroom yield for commercial growth. But unfortunately, there is no previous research about this.

However, the physical and chemical properties of logs always change during their decay process, and these changes are related to wood ear mushroom growth. The

decay process of logs can be tracked with the changes of these properties. There must be a preferred decay level for wood ear mushroom growth on Pecan logs.

During the wood decay processes, the cellulose and lignin contents are lysed by cellulase and ligninase which can be the nutrition resource for the fungus growth. The cellulase and hemicelullase activities could lead to wood degradation. Ligninase activities and acid proteinase activities are also related to wood decomposition. For more soluble carbohydrate produced, and wood substrate would be optimal for mushroom growth. Therefore, the wood would become available nutritional resource for mushroom growth during the decay process. Many researches have studied the effects of different carbon sources and nitrogen sources for mushroom growth. But there is no study about how carbon and nitrogen affect the growth of mushrooms. Based on previous research in the literature, the best carbon and nitrogen ratio of logs for mycelium growth was 35:1 for wood ear mushroom (Luo, 1993). The logs came from hardwood trees, but it was not indicated what specialized hardwood trees were used for mushroom growth. In here, it was hypothesized that wood ear mushroom starts growing on decomposed logs with specific decay level at particular C/N ratio and nutrition contents for Pecan logs.

III.2 Objectives and hypothesis

The objective was to find the preferred decay level of pecan logs for wood ear mushroom growth. Wood ear mushrooms were cultivated directly on logs with different decay levels.

It was assumed that wood ear mushroom would grow on pecan logs with specific decay level and that logs with other decay levels would have no mushroom growth.

III.3 Experimental plan for log cultivation

The overall research experiment had 3 replicates for each density category for mushroom growth in a growth chamber. The growth chamber was constructed to provide the suitable environment for mushroom growth. Logs were pecan wood and collected from Central Texas. For each replicate, there were 6 logs with densities from 200 to 800 $kg \cdot m^{-3}$ (d.w).

Table III-1. Log categories defined by densities

Log densities ($kg \cdot m^{-3}$) (d.w)	Log category
200 - 300	1
300 - 400	2
400 - 500	3
500 - 600	4
600 - 700	5
700 - 800	6

The whole chamber was divided into two parts for separate tests, and the total growth period of each batch was expected to last around 3 months based on the mushroom growth process. After 45 days from when the first batch started, the

cultivation of mushroom was started in the other side. Then after 3 months, mushroom growth in first side would be finished, and the third batch of mushroom growth would be started in this side.

The Auricularia auricula-judae spawns were bought from the online seller Bonanza in Lithuania. Logs were collected from the natural environment and cut into 1-foot lengths. They were put in an air-conditioned room for at least one week. Later, water contents, masses and volumes were measured. Eighteen logs (3 per each of the six density categories) were selected for mushroom growth. For these logs, each log had 9 holes drilled into it for spawn plugs; 3 at bottom, 3 at middle and 3 at top parts. The hole was drilled as 0.95 cm (0.375 inch) diameter and 4 cm depth.

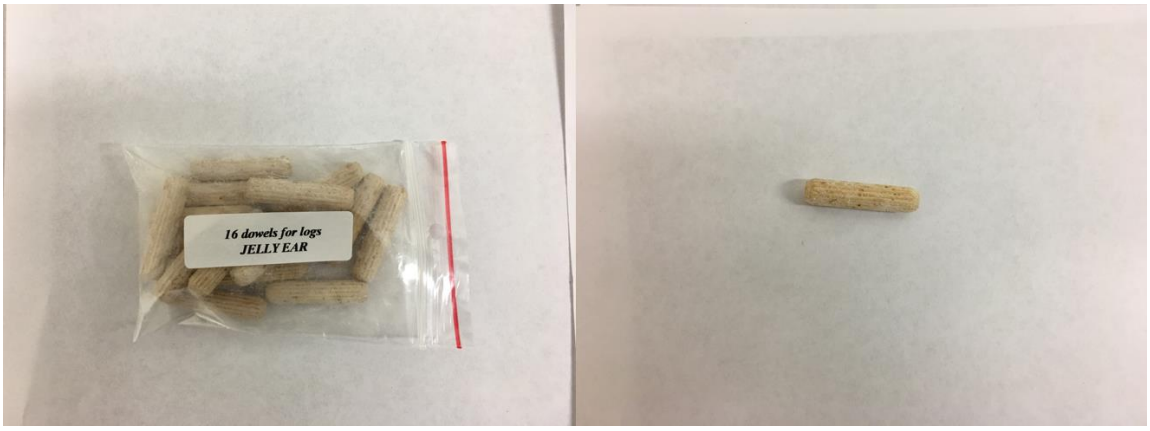


Figure III-1. Dowel spawns for wood ear mushrooms

The spawn plugs (Figure III-1) were put inside the holes and sealed with wax. It was 0.85 cm diameter and 3.8 cm length. Then, the logs (Figure II-4) were put inside the growth chamber after spawn inoculation. For mushroom mycelium development, logs

needed to be kept wet. Water containers were put on the top of the chamber to drip water to keep the logs wet so they had enough water content for mushroom growth. For fruiting body development stage, 90% of air humidity was the best for wood ear mushroom (Luo, 1993). In this research, two humidifiers were used to try to reach this level.

If the mushrooms developed fruiting bodies, the mushroom would be harvested and weighed. The mushroom fruiting bodies could be freeze dried for possible later analysis. The nutrition values, like polysaccharide, protein and energy values could be measured later. These values could have been used to evaluate the harvested mushroom and determine the preferred decay level. The polysaccharide is related to the anti-oxidative properties in aging mice (Zhang, et al., 2010) But in this research, mushroom growth stopped at the mycelium growth stage and the fruiting bodies did not develop.

Table III-2 shows the anticipated timetable for the log growing experiment.

Table III-2. Timetable of the log cultivation experiment

Activity	Implementation Time			Responsibility
1. log selection and chamber environment test	Month 1	Month 2-5	Month 6	Peiyao
	xxxxxx			
2. log physical properties measurement	xxxxxx			Peiyao
3. log chemical properties measurement	xxxxxx			Peiyao
4. Mushroom cultivation in growth chamber		xxxxxx		Peiyao
5. Harvest mushroom evaluation			xxxxxx	Peiyao

III.4 Methodology

III.4.1 Growth chamber layout

The growth chamber was designed to provide the suitable environment for wood ear mushroom growth. Figures III-2 through III-4 showed the layout of the growth chamber used in this study.

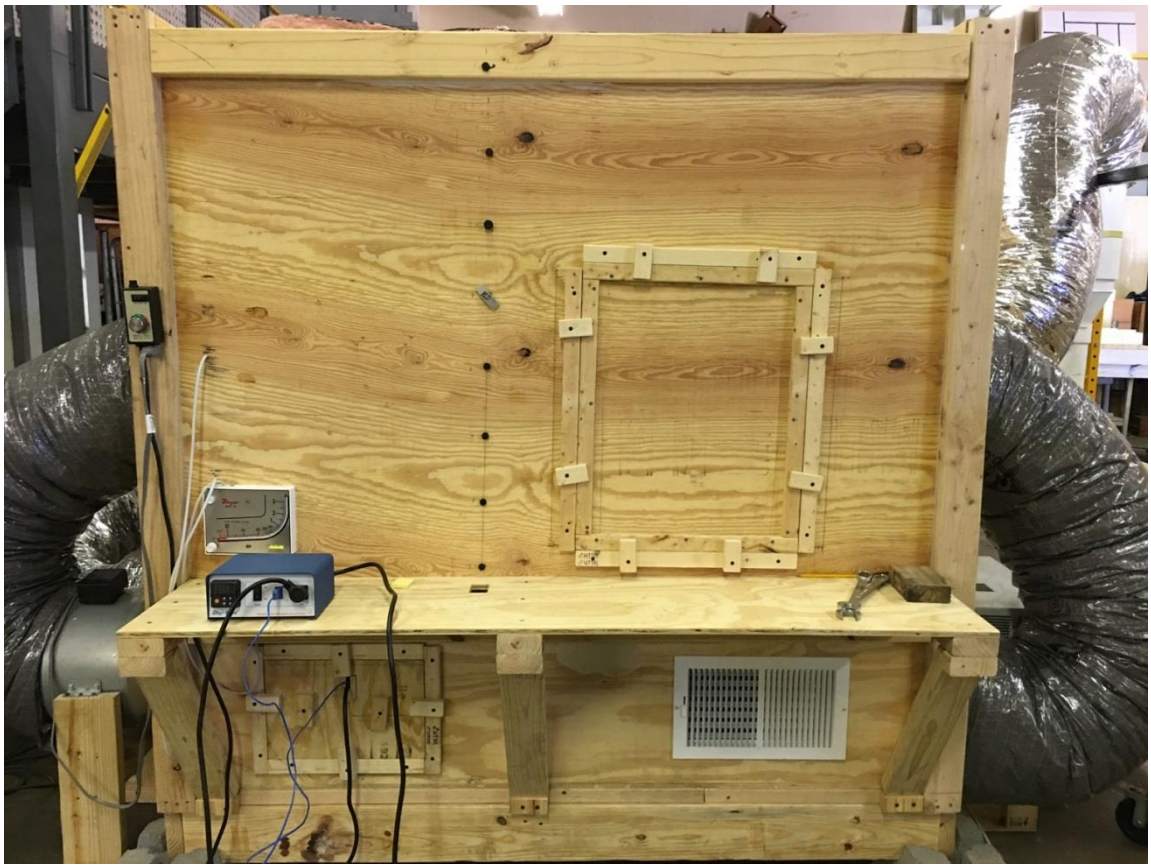


Figure III-2. The front view of growth chamber

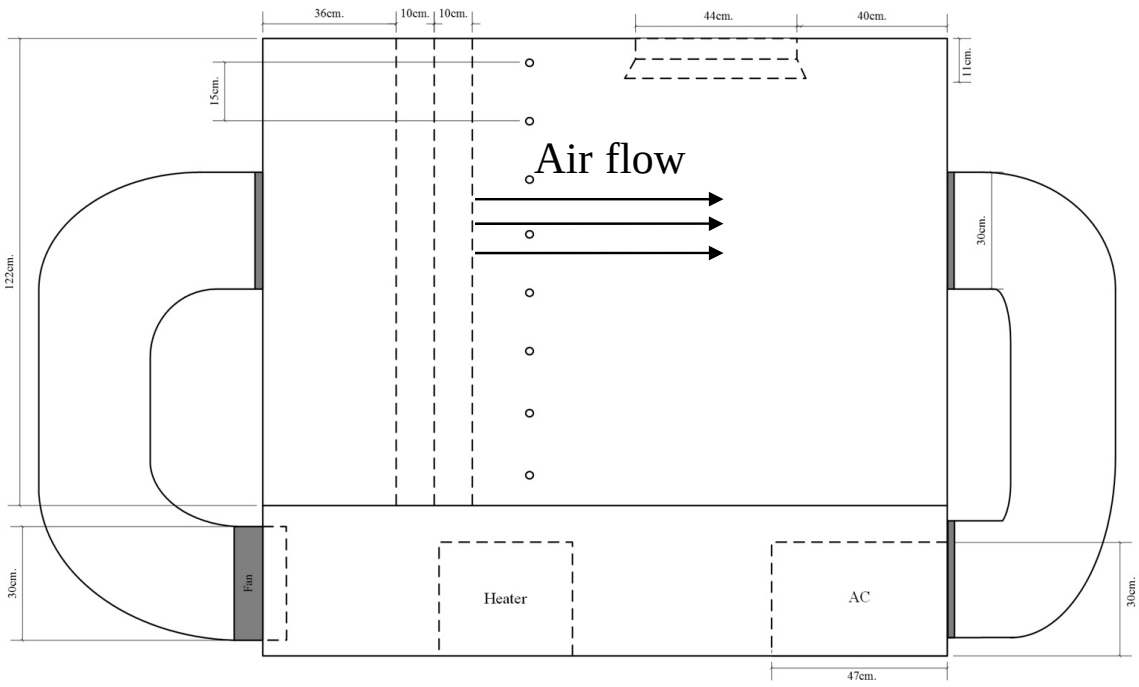


Figure III-3. Schematic of growth chamber- side view

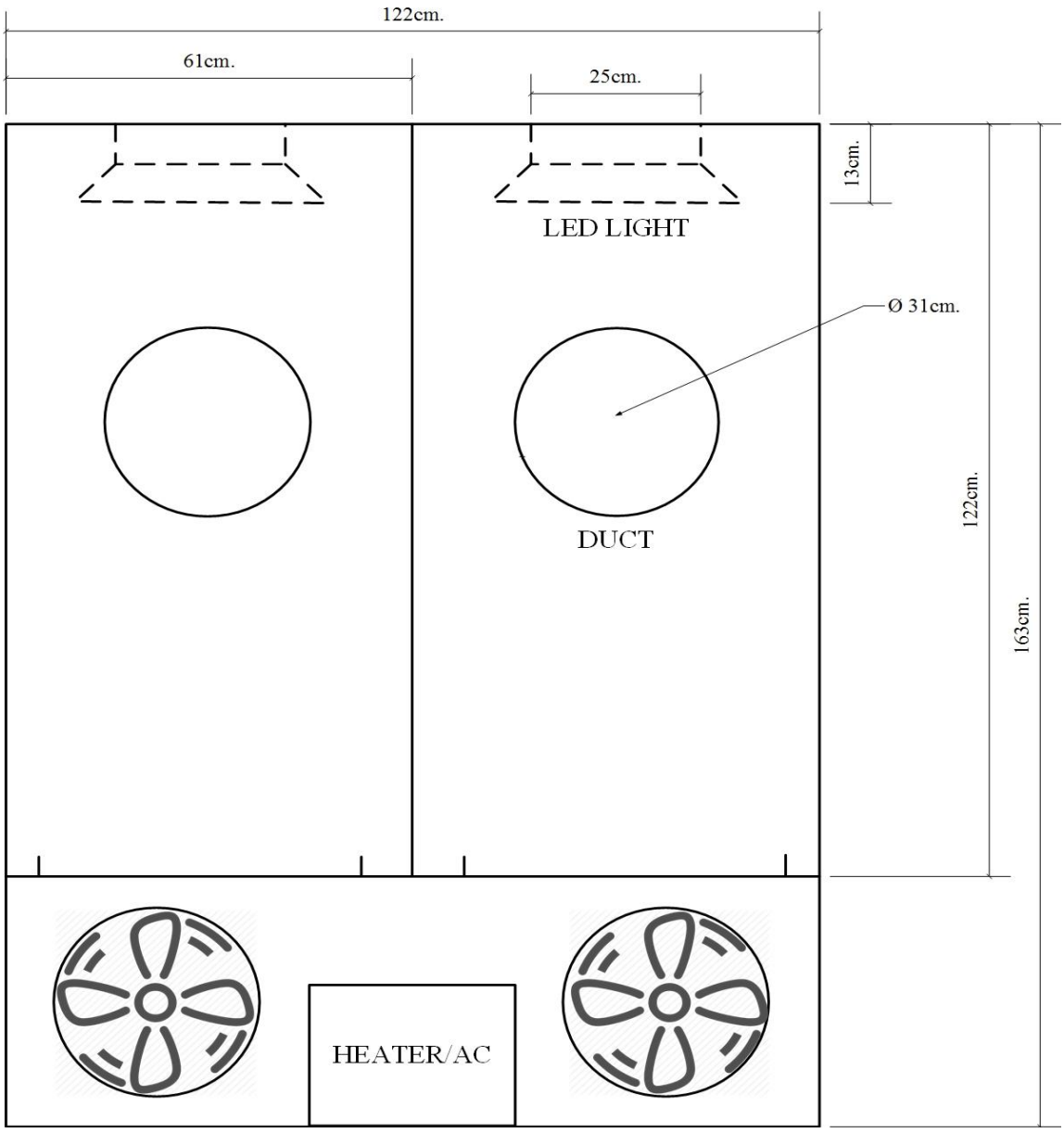


Figure III-4. Schematic of growth chamber- end view

The logs were put on trays in the chamber (Figure III-5), with 6 logs in each side. The logs were leaning against wood braces to keep them at 45° from the tray floor.

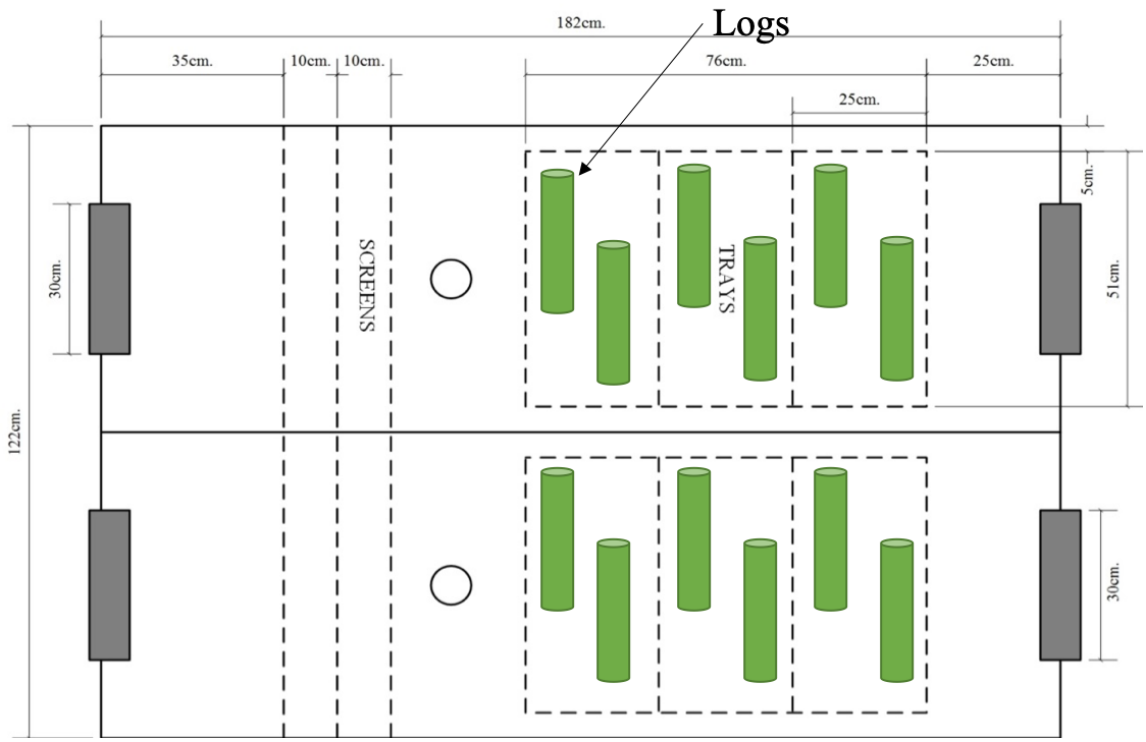


Figure III-5. Top view of growth chamber

To keep the logs with enough water content, the logs were watered after spawn inoculation. RO water was used in this research. Two 5-gallon water bottles were put on the top of the chamber on both sides and connected with water pipes which dripped water onto the logs inside the chamber, Figure III-6.

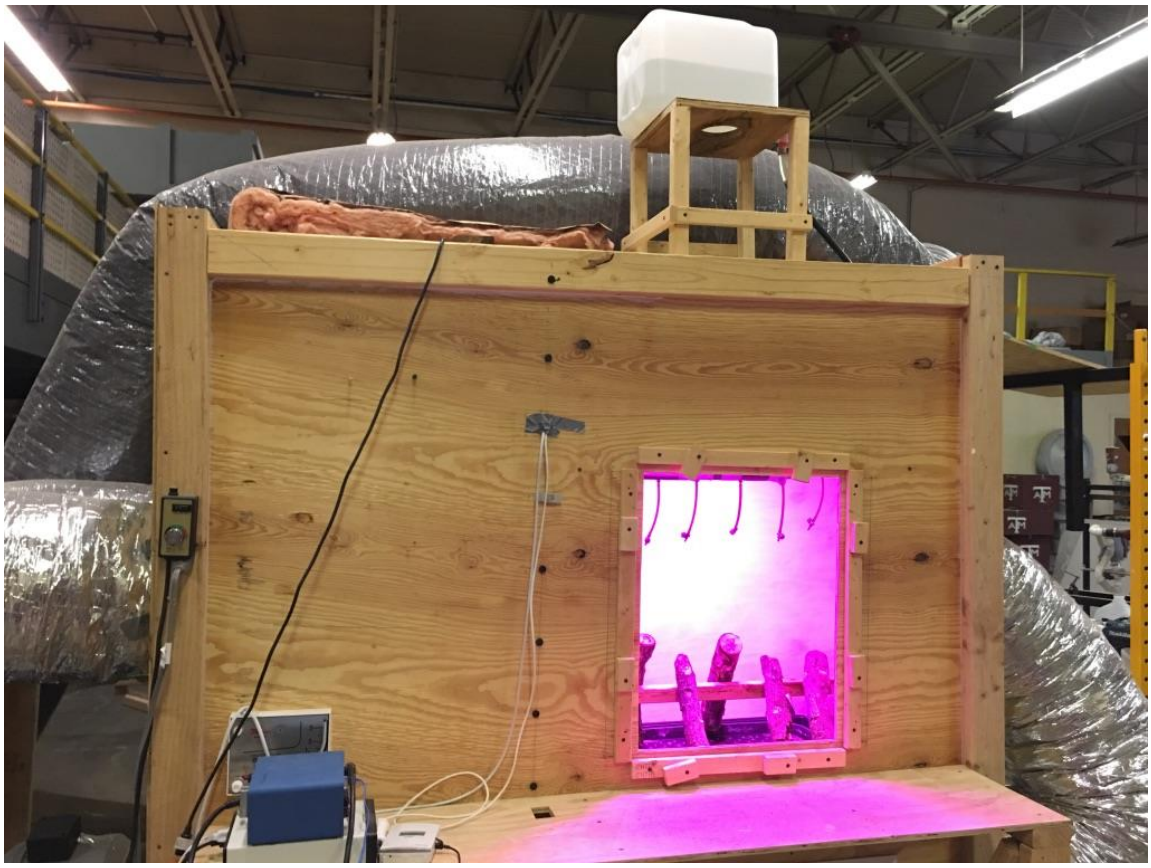


Figure III-6. The water dripping of the growth chamber

III.4.2 Growth chamber environment control

All mushrooms were cultivated in the growth chamber under controlled environment, such as light, temperature, relative humidity and air velocity. All the physical environment conditions for both the log cultivation method and the bag cultivation methods were the same.

In this research, the temperature was controlled at range of 22-26℃ in the growth chamber, Figure III-21 and III-23. The temperature was controlled by an air conditioner, heater and heat controller (Digi-Sense, TC5000). The air conditioner was set at 22℃ and

the heater was controlled by a controller set at 28℃. The sensor of the TC5000 was placed on the bottom part of growth chamber. Hence, the temperature in the chamber had been monitored to be around 24℃ which was suitable for mycelium growth (Luo, 1993), Figure III- 21 and Figure- 23. After mycelium growth was finished, the temperature was adjusted to 25 ℃ for fruiting body growth, Figure III- 21 and Figure- 23.

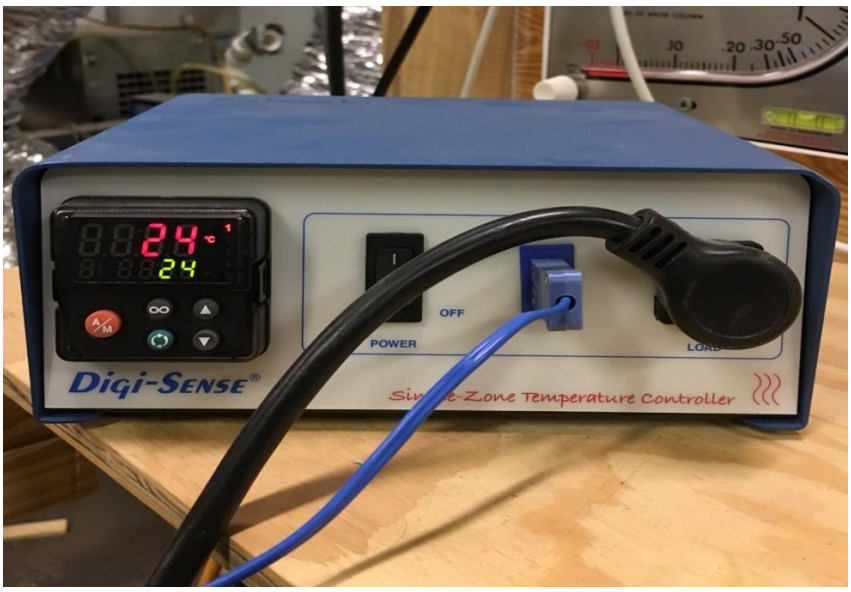


Figure III-7. Digi-Sense PID temperature controller (Digi-Sense, TC5000)

For the relative humidity, there were two humidifiers and a humidity controller (ETS, 5200) to provide stable humidity. Distilled water was used for the humidifier. Since mushroom growth needed very high relative humidity, the controller was set at 90% (Luo, 1993). However, the relative humidity varied from 30 to 90 % inside growth chamber, Figure III- 22 and III- 24. So logs were watered every day to keep it wet. The sensor of the controller was placed inside the growth chamber in the bottom part.

Figure III-8. Humidifier and Microprocessor Controller (ETS, 5200)

The temperature and relative humidity inside the chamber and the outer room were measured during the same time. For room temperature and humidity, a Campbell scientific CR3000 datalogger and Vaisala HMP155A probe were used for the recording. The HMP155A probe was placed on the desk beside the growth chamber to record the data of room temperature and humidity.

Figure III-9. Micrologger (Campbell, CR3000) and HMP155A probe (Vaisala, HMP155A)

To measure the temperature and humidity inside the growth chamber, two onsite Hobo data loggers were used and sensors were put in both sides of the growth chamber. The temperatures and relative humidities of all equipment were visually observed every day.

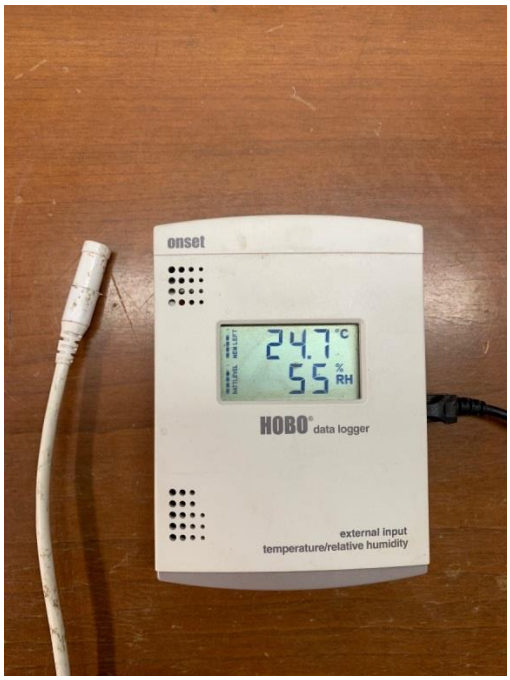


Figure III-10. Hobo data logger (Hobo, U12 series)

Air was circulated by a Fantech 12 in. (30.48 cm) inline centrifugal duct fan. The fan had 6 different control settings from high to low speed, denoted as S1 to S6. In this experiment, it was set at S4. The pressure difference was -0.04 in. of water column between inside and outside of chamber when the fan was running.

To keep a stable and uniform air flow, three perforated metal screens were installed at 4 in. (10.16 cm) apart from each other and 10 in. (25.4 cm) from the

chamber end, Figure III-11.

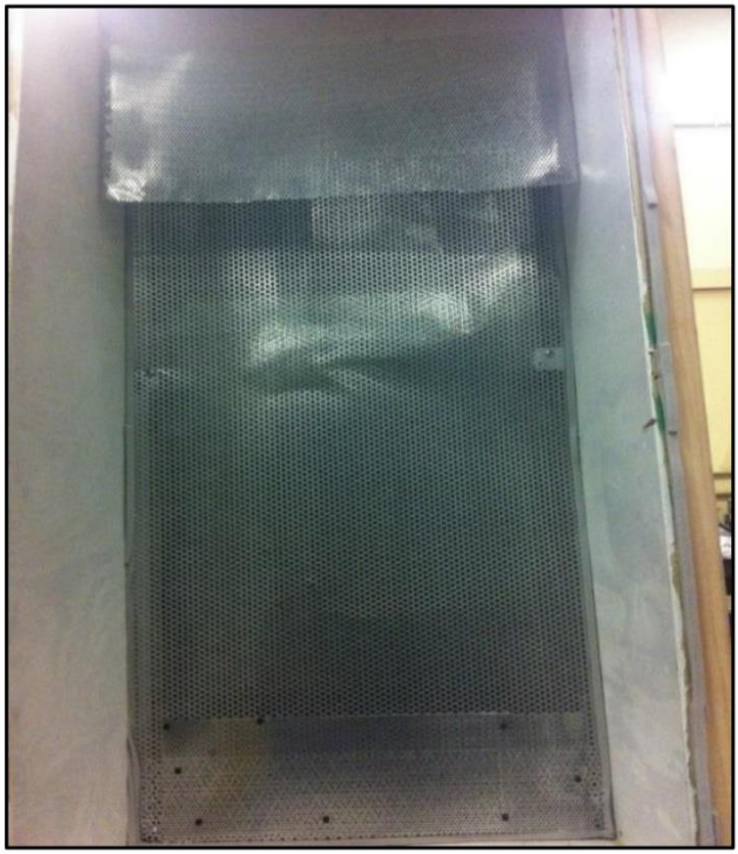


Figure III-11. Perforated metal screens (Li Ge, 2015)

A TSI VelociCalc air velocity meter (TSI, 9515) was used to measure the air velocity inside the chamber. The accuracy was $\pm 0.5\%$ of reading. A 6×8 grid between filter screen and trays was used for velocity measurements through pre-drilled holes on the side wall. The column distance was 10.16 cm (4 in.) and row distance was 15.24 cm (6 in.) for the grid.

Figure III-12. The grid holes for air velocity uniformity measurement and TSI VelociCalc air velocity meter (TSI, 9515)

Table III-3 showed the air velocity ($m \cdot s^{-1}$) for all grid positions in the chamber. The heading of each row and column represented the distance (m) of measurement position to the side wall and floor.

The average velocity for the left side was 0.34 $m \cdot s^{-1}$and the standard deviation was 0.100. For the other side of the chamber, the average air velocity was 0.36 $m \cdot s^{-1}$, and standard deviation was 0.188.

Table III-3. Air velocity ($m \cdot s^{-1}$) distribution inside growth chamber of left side, the heading of the row and column represent the distance (m) to the left-side wall and floor of growth chamber

	0.5	0.15	0.25	0.36	0.46	0.56
1.14	0.41	0.39	0.50	0.39	0.44	0.42
0.99	0.62	0.31	0.30	0.26	0.26	0.47
0.84	0.36	0.25	0.41	0.37	0.41	0.46
0.69	0.46	0.49	0.52	0.47	0.37	0.09
0.53	0.27	0.34	0.18	0.25	0.26	0.27
0.38	0.33	0.29	0.29	0.27	0.25	0.21
0.23	0.34	0.32	0.42	0.28	0.29	0.26
0.08	0.40	0.41	0.36	0.26	0.25	0.31

Table III-4. Air velocity ($m \cdot s^{-1}$) distribution inside growth chamber of right side, the heading of the row and column represent the distance (m) to the right-side wall and floor of growth chamber

	0.5	0.15	0.25	0.36	0.46	0.56
1.14	0.44	0.41	0.16	0.22	0.24	0.20
0.99	0.53	0.43	1.33	0.47	0.41	0.43
0.84	0.62	0.23	0.66	0.57	0.37	0.36
0.69	0.5	0.44	0.56	0.5	0.47	0.32
0.53	0.21	0.27	0.27	0.25	0.26	0.26
0.38	0.21	0.23	0.24	0.22	0.26	0.21
0.23	0.26	0.34	0.36	0.3	0.34	0.26
0.08	0.36	0.37	0.24	0.29	0.26	0.25

For light requirements for mushroom growth, a diffused small amount of light was used to facilitate mycelium growth. During the fruiting body development stage, light with 500 lux intensity was provided.

A LumiGrow ES330 LED light (Figure III-13) was used in each side of the growth chamber to provide the light for mushroom growth. LED has many advantages compared to incandescent lights and compact fluorescents, such as consistent light quality, energy conservation, operating cost savings, reduced maintenance and spectral control.

Figure III-13. LumiGrow ES330 LED light, *reprinted from The Hydro Source*. Retrieved 2013, from http://www.thehydrosource.com/lumigrow-es330.html

For this research, the LED lights were using blue (430 - 480 nm) and red (480 – 630 nm) wavelengths spectrum in the controlled environments. The LumiGrow LED fixture produced light about 17% in the blue region and 67% in the red region and 10% between the red and blue regions (Figure III-14), when it reached maximum light intensity.

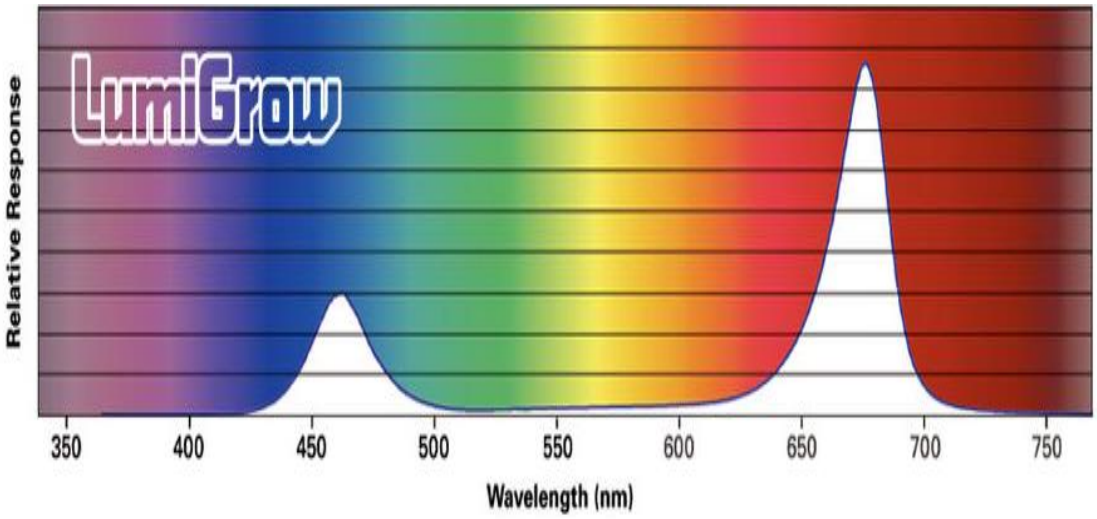


Figure III-14. LumiGrow ES330 light color spectrum, *from LumiGrow Inc*, 2014

During fruiting body development period, 500 lux light intensity was required. To adjust the light intensity, a Li-Cor quantum LI190SB light sensor (Figure III-15) was used. The unit of Li-Cor was PPFD, and it converted to lux to compare with the previous research. According to the manual of the LI190SB based on Campbell Scientific Company, the sensor measures PAR in 400 to 700 nm waveband. And the sensor is designed to measure PAR received on a plane surface.

Figure III-15. Li-Cor quantum light sensor, *reprinted from Li-Cor Inc*. From https://www.licor.com/env/products/light/quantum.html

III.5 Log cultivation process of wood ear mushroom

III.5.1 Growth process in chamber

The growth chamber was divided into two sides labelled A and B. The log cultivation of the first batch started from January 15, 2018 in side A. After one and half months, the second batch of logs started in side B. The third batch started after 3 months from first batch. The anticipated growth time for each batch was 3 months.

Figures III-16 through III-19 showed photos of the logs at different growth periods in the growth chamber. The numbers in the figures represented the categories of each logs based on densities. Figure III-16 showed the logs just after the spawn plugs were put inside the logs. Each log was injected with 9 spawn plugs. The plug holes were sealed by wax and duct tape. During this growth period, the logs were put inside the growth chamber. Only low lux intensity of blue and red light around 300 lux was given to induce the mycelium growth and the light intensity was measured by Li-Cor light sensor. Logs were watered with RO water 2 or 3 times every week depending on log moisture conditions. The temperature was controlled at 23-25℃. Relative humidity was measured to be around 60-75% inside the growth chamber.

Figure III-16. Logs just after they were inoculated

After one month of spawn incubation, it was estimated that the mycelium could be detected in some of the logs. The following pictures were logs after one month of inoculation. From the Figure III-17, there was some white mycelium that appeared on logs 1, 2 and 4. The density categories of these two logs were 1, 2 and 4.

Figure III-17. Logs after one month of spawn inoculation

Two months later, there were still some mycelium on logs 1, 2, 3, and 4. But the fruiting bodies didn't yet appear. The density categories were 1, 2, 3 and 4 of logs with mycelium.

Figure III-18. Logs after two months of spawn inoculation

After 3 months of spawn inoculation, the fruiting bodies still didn't appear and some mycelium became dark. It seemed that the mycelium stopped growing after 3 months.

Figure III-19. Logs after three months of spawn inoculation

III.5.2 Logs with known mushroom fruiting body residue that were regrown in the growth chamber

To find the reason why fruiting body didn't appear on logs and whether the environmental condition was suitable for fruiting body development in the growth chamber, pecan logs with known mushroom residue (dried fruiting body) were put in the growth chamber and regrew for further investigation later.

There were 6 pecan logs that were collected from the natural environment which had known residue of wood ear mushroom fruiting bodies. The pre-treatment was also used for these logs. They were put in a lab room with 24 ℃ temperature and 40 % moisture content for one week.

The physical and chemical properties of these logs were measured as described previously. These logs were then placed in the growth chamber. The environmental conditions were kept the same as previously described for the fruiting body development stage. Some new fruiting bodies of the wood ear mushrooms appeared on one log after one and half months (Figure III-20). Consequently, the conditions that the logs were exposed to within the growth chamber were sufficient to produce fruiting bodies of pre-inoculated pecan logs.

There were another 7 logs that were collected from the natural environment with mushroom residue on them. These 7 logs were not put in the growth chamber. The physical and chemical properties of these logs were also measured. Hence, 13 logs with mushroom residue were collected totally.

Figure III-20. Log from natural environment with mushroom residue after one and half month with new mushroom growth

III.6 Results and Discussion

III.6.1 Environmental conditions in the growth chamber and ambient environment

Figures III-21 through III-26 showed the relative humidity and temperature inside the growth chamber. And the temperature and relative humidity was logged every 5 minutes. The numbers on the horizontal axis represent the time. The temperature of the left side changed between 20 and 25 ℃ which was suitable for mushroom growth, Figure III-21.

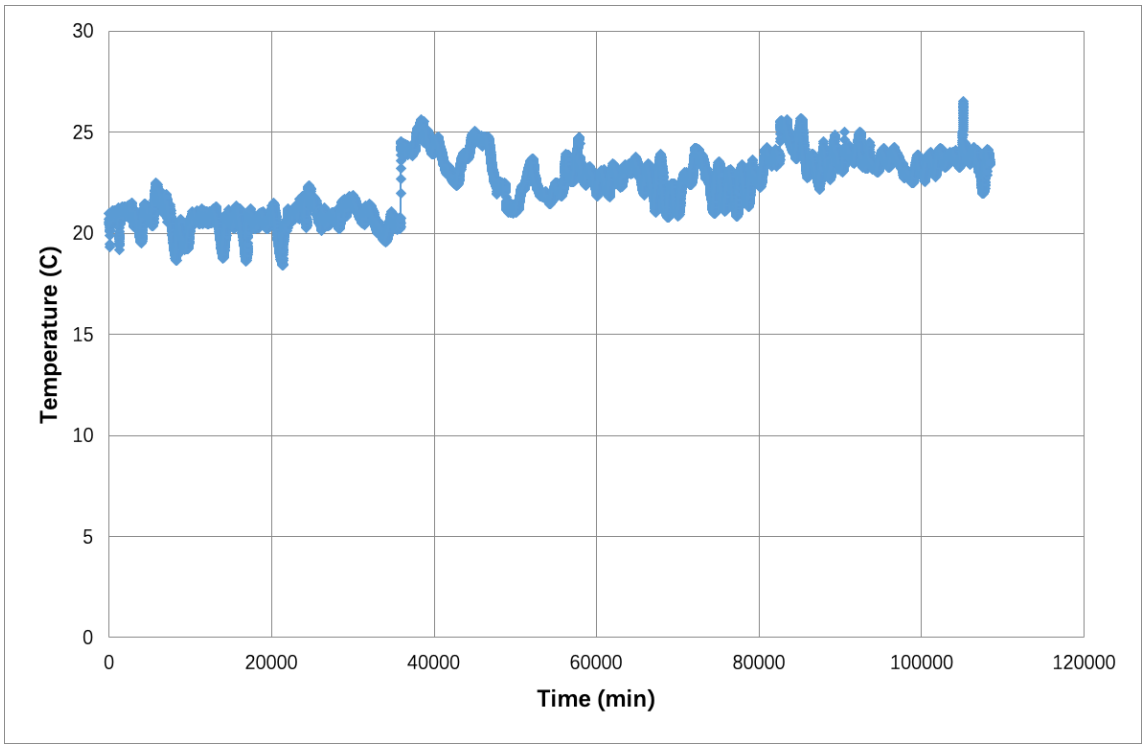


Figure III-21. The temperature within the left side of the growth chamber

The relative humidity varied from 30 to 80%, Figure III-22. During the mycelium growth period, mycelium could grow at low relative humidity. But at the fruiting body stage, it needed high relative humidity above 80%. However, the logs were watered every day to keep logs wet during fruiting body development.

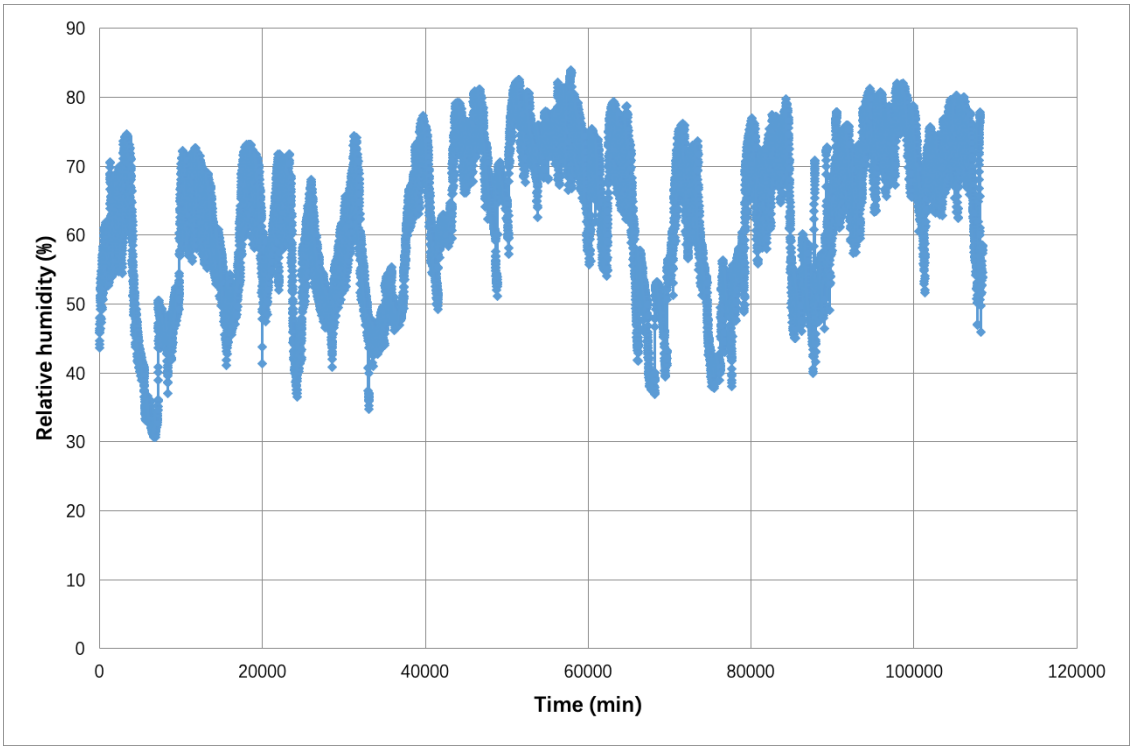


Figure III-22. Relative humidity of left side inside growth chamber

For the right side of growth chamber, temperature changed between 20 and 25 ℃ during the most of time. Figure III-23. The growth of mycelium and fruiting body were comfortable at this range.

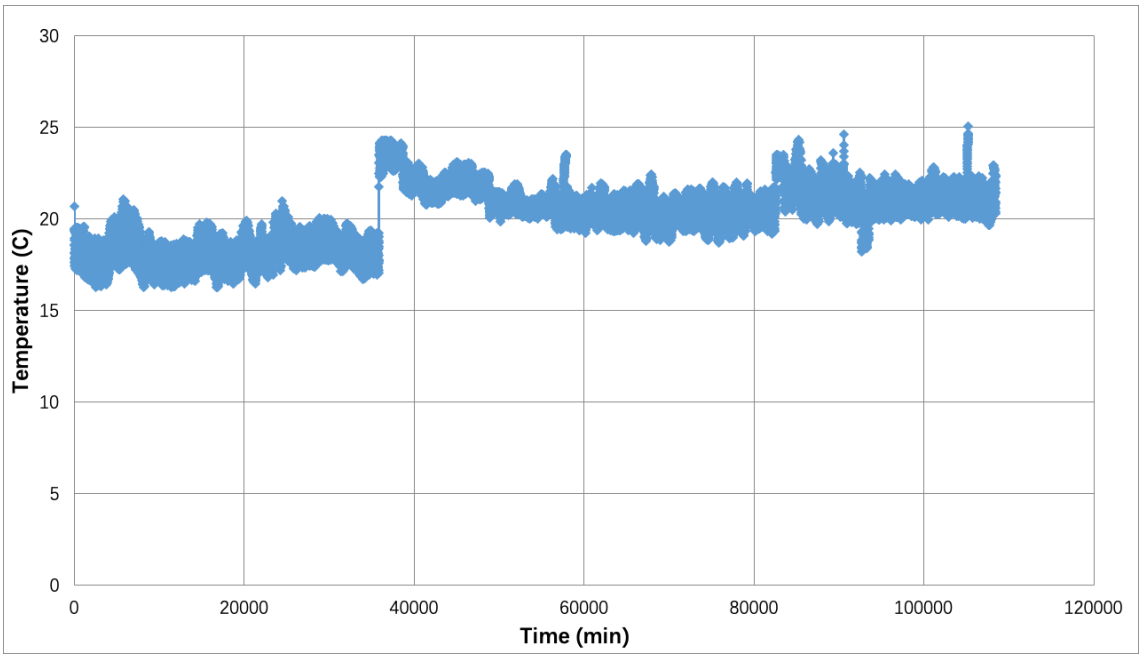


Figure III-23. Temperature of right side inside growth chamber

Relative humidity varied from 35 to 90 %, Figure III-24. For fruiting body development, it was required to be above 80 %. So logs were watered to keep it wet for mushroom growth.

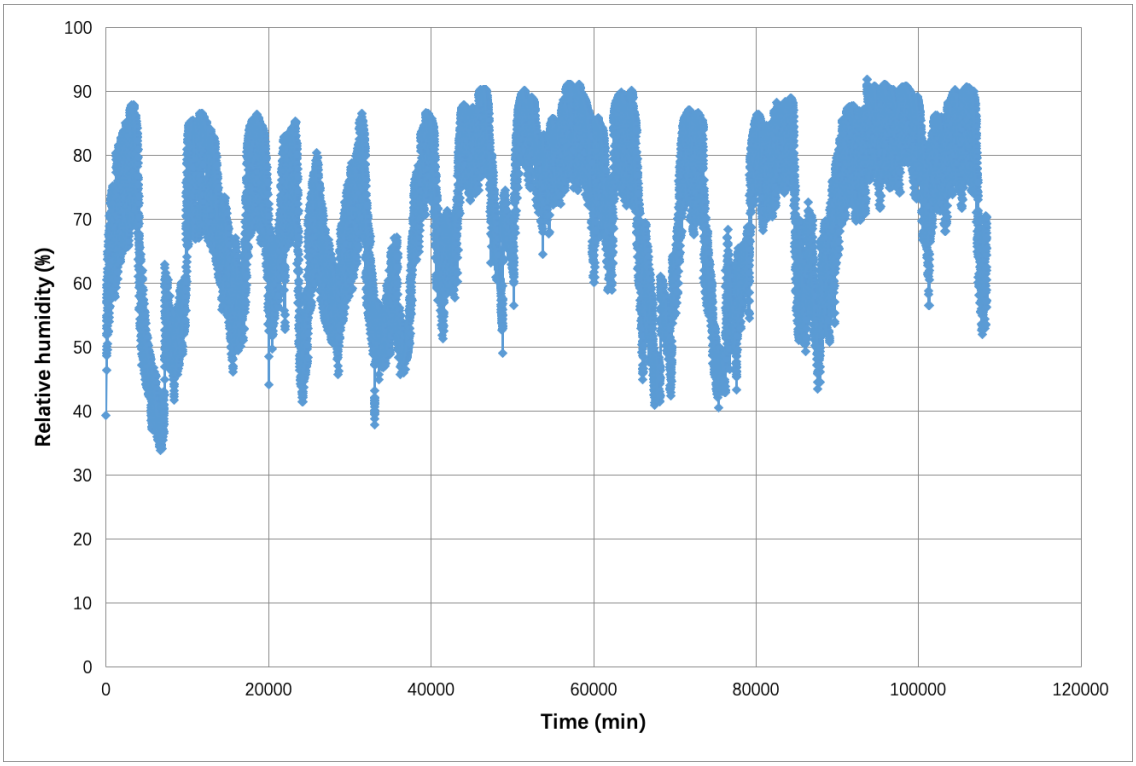


Figure III-24. Relative humidity of right side inside growth chamber

Ambient temperatures outside of the growth chamber varied from 16 to 23 ℃ during winter, Figure III-25.

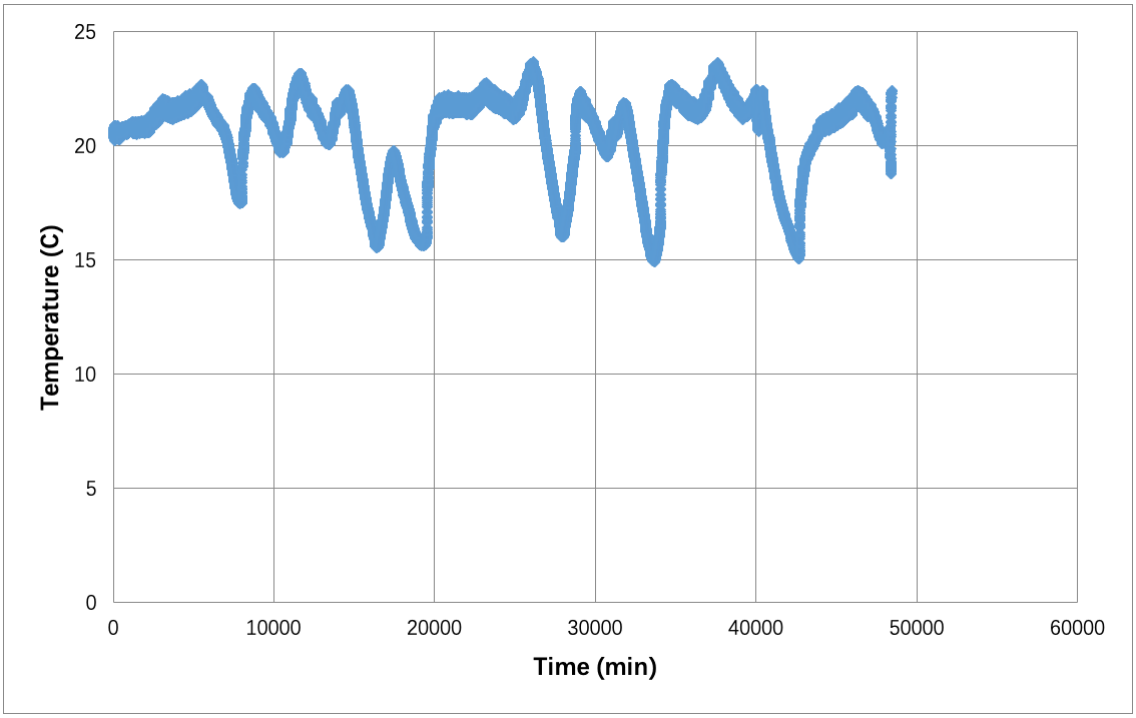


Figure III-25. Temperature outside of growth chamber

However, ambient relative humidity varied from 30 to 70 % during the winter time, Figure III-26. It was pretty dry at most times.

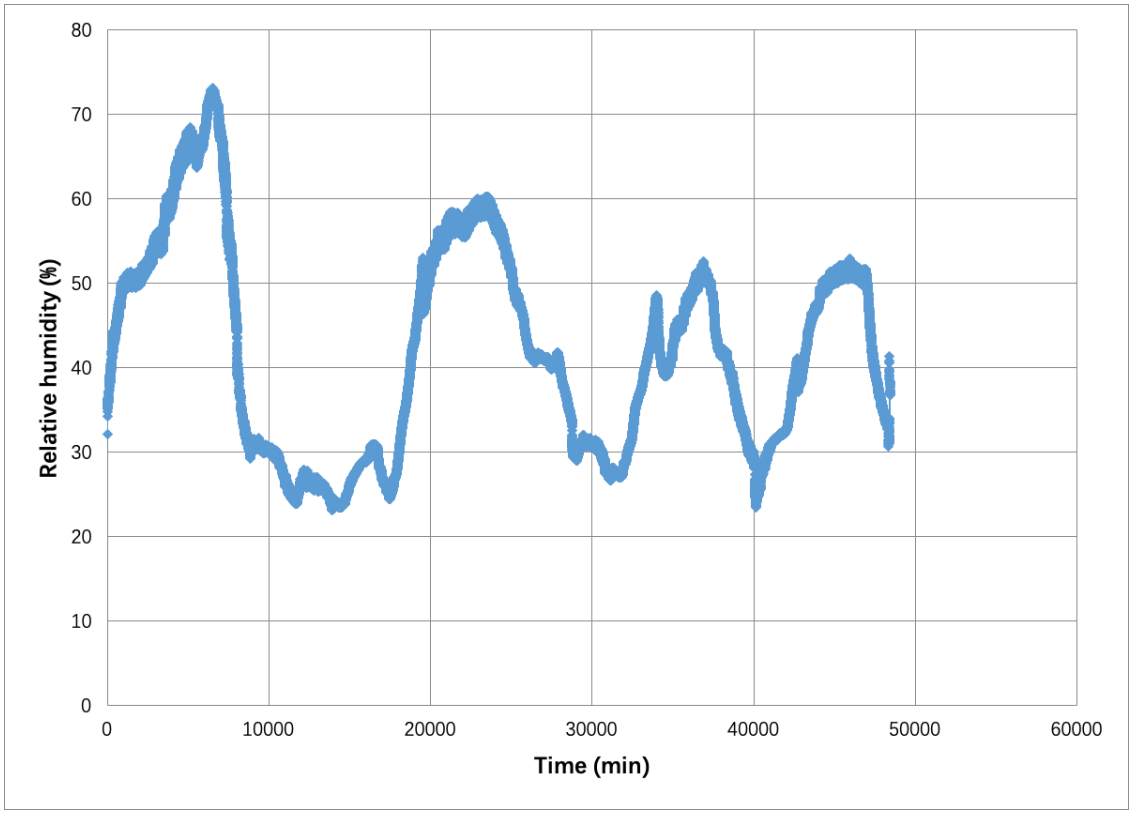


Figure III-26. Relative humidity outside of growth chamber

III.6.2 Mycelium and fruiting body

For log cultivation of wood ear mushroom, some white mycelium appeared on some logs with density from 200 to 600 $kg \cdot m^{-3}$ at category 1, 2, 3 and 4. And the C/N ratios were around 60 to 90 for these logs. But the fruiting body didn't develop.

III.6.3 The physical and chemical properties of logs with known residue of mushroom fruiting bodies

For the 6 logs with known mushroom fruiting body residue put in the growth chamber, only one log grew new wood ear mushroom on it. The data of physical and chemical properties were measured, Table III-5. The density and C/N ratio of the log which grew new mushrooms was 502 $kg \cdot m^{-3}$ and 74.75. The density was in Category 4 from 500 to 600 $kg \cdot m^{-3}$. The densities of all logs (total of 13 logs) with mushroom residue varied from 200 to 600 $kg \cdot m^{-3}$, which put them in log density categories of 1, 2, 3 and 4.

Table III-5. The data of physical and chemical properties of 13 pecan logs with known mushroom fruiting body residue based on density categories

category	Density range ($kg \cdot m^{-3}$)	Density Avg ($kg \cdot m^{-3}$)	Density SD	Logarithm of Penetration depth avg. (mm)	Penetration SD	C/N Avg	C/N SD
1	200-300	255.00	3.51	0.76	0.40	172.52	117.53
2	301-400	309.85	0.77	0.39	0.08	137.51	45.58
3	401-500	428.88	17.66	0.14	0.01	110.88	35.09
4	501-600	536.10	30.64	0.16	0.03	61.47	15.22
5	601-700						
6	701-800						

Figure III-27 showed the relationships between penetration and density of logs with mushroom residue. The P- value was 0.0032 which was less than 0.05, Table III-6. Hence, the linear relationship was significant. There was a negative relationship between density and penetration of logs with mushroom residue.

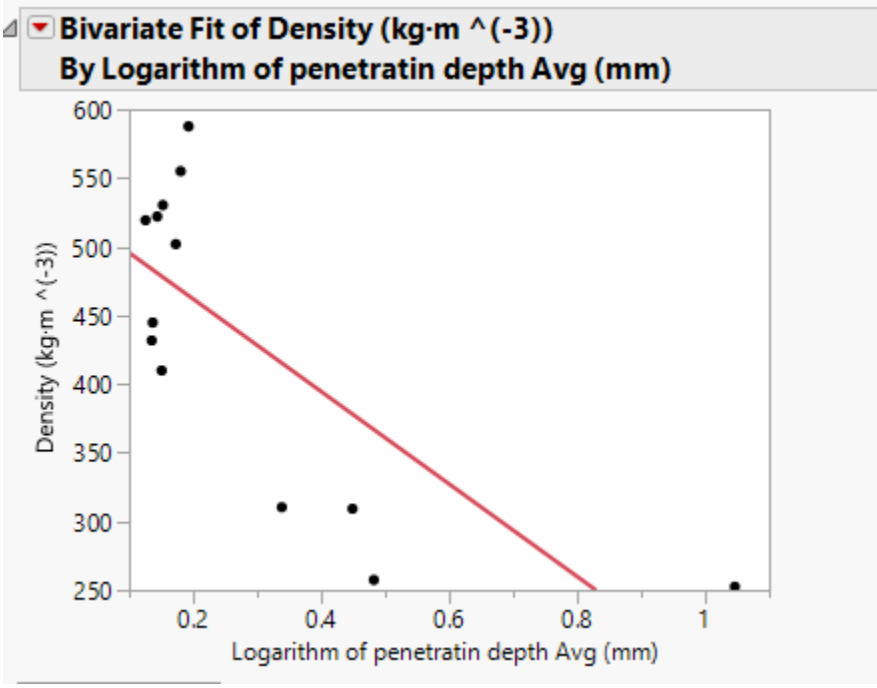


Figure III-27. Density versus logarithm of penetration depths of 13 logs with residues

Table III-6. ANOVA analysis of density versus penetration of 13 logs with residues

Summary of fit	RSquare	0.56
	Rsquare Adj	0.52
	Root Mean Square Error	80.59
	Mean of Response	433.30
	Observations (or Sum Wgts)	13
Analysis of Variance	F Ratio	14.08
	Prob. > F	0.0032

The plot indicated that carbon contents decreased with increasing densities, Figure III-28. However, P-value was 0.07 which was larger than 0.05, Table III-7. Hence, the relationship was not significant. The carbon content increased slightly during log decay process.

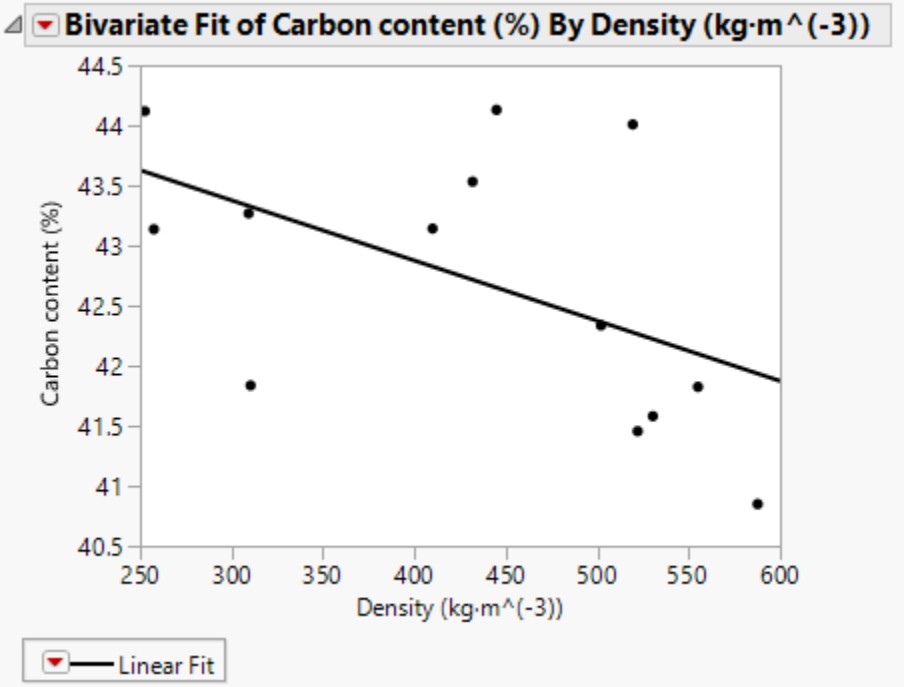


Figure III-28. Carbon content versus densities of 13 logs with mushroom residue

Table III-7. The ANOVA analysis of density versus carbon contents of 13 logs with residue

Summary of fit	RSquare		0.27
	Rsquare Adj		0.21
	Root Mean Square Error		0.99
	Mean of Response		42.71
	Observations (or Sum Wgts)		13
Analysis of Variance	F Ratio		4.11
	Prob > F		0.07
Parameter Estimates	Intercepts	Estimate	44.88
		t Ratio	40.57
		Prob > |t|	<0.0001
	Density	Estimate	-0.005
		t Ratio	-2.03
		Prob > |t|	0.07

There was a positive relationship between nitrogen contents and densities. The nitrogen contents increased with increasing densities, Figure III-29. The P-value was 0.0037. Hence, the relationship was significant. The nitrogen content decreased significantly during log decomposition.

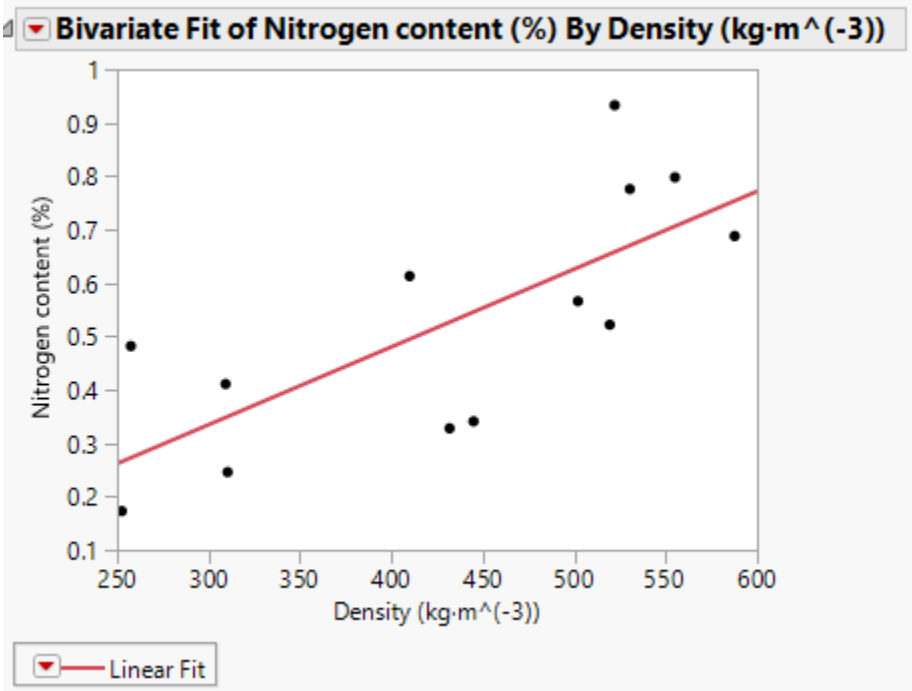


Figure III-29. Nitrogen contents versus densities of 13 logs with mushroom residue

Table III-8. The ANOVA analysis of densities versus nitrogen contents of 13 logs with mushroom residue

Summary of fit	RSquare		0.55
	Rsquare Adj		0.51
	Root Mean Square Error		0.16
	Mean of Response		0.53
	Observations (or Sum Wgts)		13
Analysis of Variance	F Ratio		13.42
	Prob > F		0.0037
Parameter Estimates	Intercepts	Estimate	-0.10
		t Ratio	-0.57
		Prob > |t|	0.58
	Density	Estimate	0.001
		t Ratio	3.66
		Prob > |t|	0.0037

From figure III-30, a linear fit was satisfied for the relationship between density and C/N ratio for these logs with mushroom residue. The P-value was 0.0061 which was less than 0.05, Table III-9. The linear relationship was significant and it was negative relationship. The C/N ratio increased during log decay process.

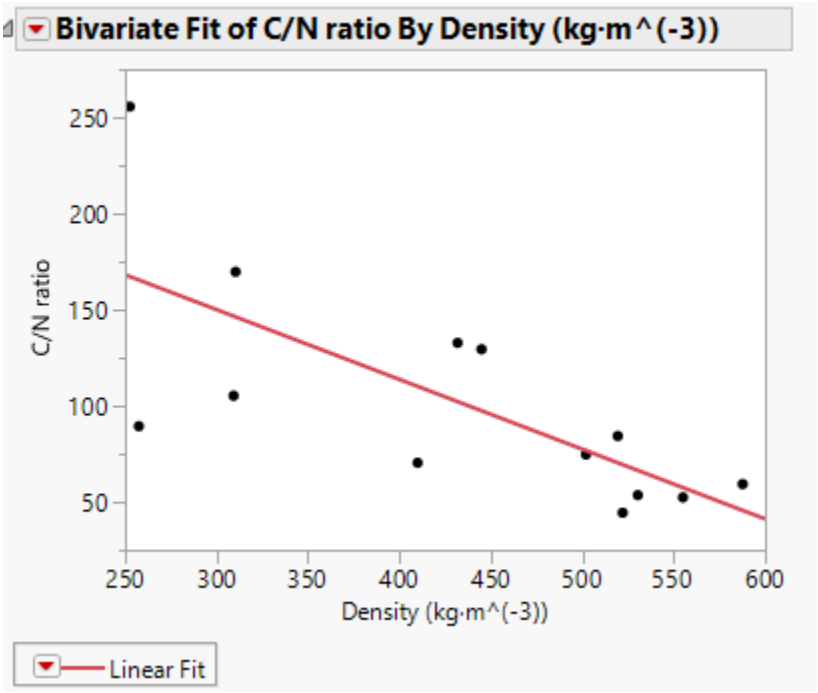


Figure III-30. Density versus C/N ratio of 13 logs with mushroom residue

Table III-9. ANOVA analysis of density versus C/N ratio of 13 logs with residue

Summary of fit	RSquare		0.51
	Rsquare Adj		0.47
	Root Mean Square Error		43.27
	Mean of Response		101.66
	Observations (or Sum Wgts)		13
Analysis of Variance	F Ratio		11.46
	Prob > F		0.0061
Parameter Estimates	Intercepts	Estimate	258.93
		t Ratio	5.40
		Prob > |t|	0.0002
	Density	Estimate	-0.36
		t Ratio	0.11
		Prob > |t|	0.0061

III.7 Conclusions

III.7.1 The preferred decay level of Pecan logs for mushroom growth

For log cultivation of wood ear mushroom, some white mycelium appeared in some logs within Category 1, 2 3 and 4 (density range from 200 to 580 $kg \cdot m^{-3}$). The fruiting body didn't appear. However, it indicated that logs at low density categories had more mycelium growth compared to logs at high density categories.

For the 6 logs out of the 13 logs with mushroom fruiting residue, that were put inside the growth chamber, only one log grew some new wood ear mushroom fruiting bodies. This log had a density of 502 $kg \cdot m^{-3}$ at Category 4 and C/N ratio of 74.75. For all the logs with known mushroom residue, the average density was 517.85 $kg \cdot m^{-3}$, and standard deviation of density was 60.55. The average value of C/N ratio was 59.15 and standard deviation was 11.52. Hence, this would suggest that log density of around 400 – 550 $kg \cdot m^{-3}$ at category 3 to 4 and C/N ratio at 60 – 80 were preferred for wood ear mushroom growth.

III.7.2 The relationship between wood properties and decay levels of 13 logs with mushroom residue

The negative linear relationship also existed between density and logarithm of penetration depths. This relationship was the same as the previous 104 logs. Therefore, for all logs, the negative relationship between density and logarithm of penetration depth was significantly existed.

For the carbon content, it decreased with increasing density of 13 logs. It indicated that carbon contents increased during log decomposition process. This relationship was the same as for 18 logs selected for mushroom growth.

The nitrogen content increased with increasing density of the 13 logs. This relationship was significant for P-value less than 0.05. The nitrogen content decreased during log decay process. And the nitrogen content also decreased during the decay

process for the 18 logs. Same relationships existed between nitrogen content and density for the 13 logs and 18 logs.

The C/N ratio of the 13 logs decreased with increasing density and this relationship was significant. It indicated that C/N ratio increased during the log decomposition process. This trend was the same compared to the 18 logs selected for mushroom growth.

Therefore, the same relationship existed between chemical properties and decay levels of the 18 logs used for mushroom growth and the 13 logs with mushroom fruiting residue.

CHAPTER IV

BAG CULTIVATION OF WOOD EAR MUSHROOMS

IV.1 Introduction

Bag cultivation of mushrooms is a simple and safe method to grow mushrooms. By using this method to grow wood ear mushroom under the required environments, the nutrition and environment conditions can be controlled and monitored. All bags were bought from the Out-Grow online sellers (Out-Grow company). The Out-Grow company is an online seller that provides spawn and liquid cultures of fungus and growth equipment for mushroom cultivation. And it also sells the mushroom products for customers. The sterilized bags can prevent infection of other fungus during mushroom growth.

The substrates needed to grow mushrooms have been divided into two layers inside the bags. For the control group, there were two kinds of growth bags. For the first type, the bottom layer was rye berry which was used for spawn incubation. And the top layer was animal manure compost. For the second type, the bottom was also rye berry, but the top was hardwood waste. Both types were purchased from Out-Grow company.

Figure IV-1. Type 1(left) and type 2 (right) growth bag of control groups

Compared to the control group, the bottom part of the experimental group was also rye berry, but the top part was wood waste made from pecan logs that were chipped and ground. Hence, the pecan surface and interior wood were mixed in the top part. All the substrates were sealed in bags with a 0.2-micron filter patch. This filter patch could be used as air exchange for the mushroom growth. The bottom of the bag had a self-healing injection port which was used for spawn inoculation. Figure IV-2 showed the growth bag of the experiment group with only rye berry. After the mycelium colonization, pecan wood substrates were added into the bags.

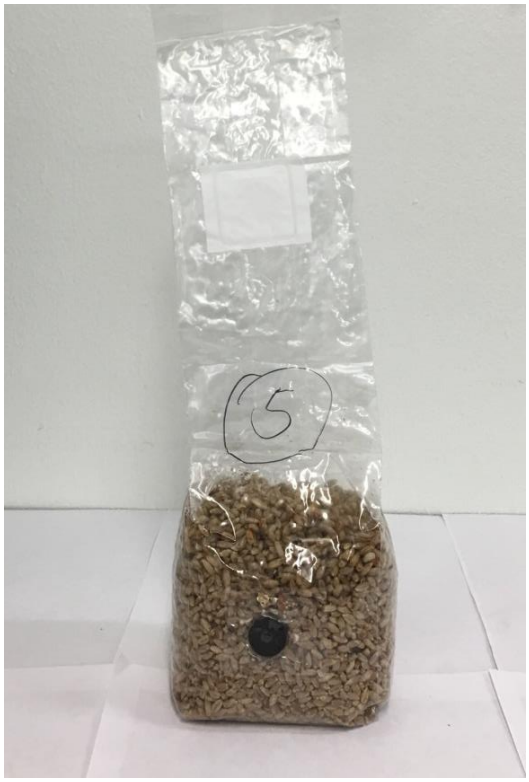


Figure IV-2 Growth bags of experiment group showing only rye berry

IV.2 Objectives and hypothesis

The objective was to grow wood ear mushrooms in bags and find the preferred category of pecan wood substrate for mushroom growth. Therefore, the decay level of the pecan wood substrate could be determined for wood ear mushroom growth. The wood substrate was made from pecan logs that were discussed earlier which were chipped and ground into the substrate. This could tell the mushroom producers which decay level of pecan wood was suitable for wood ear mushroom growth.

The hypothesis was that wood ear mushrooms prefer to grow in bags with a specific substrate and these substrates are made by pecan wood at certain decay levels.

IV.3 Experimental plan of bag cultivation of wood ear mushrooms

After the log cultivation of mushrooms, the growth chamber was used for bag cultivation of wood ear mushrooms. The experiment had 3 replicates at each density category, and logs at different density categories from 200 to 800 $kg \cdot m^{-3}$ were ground to wood chips as substrate for bag cultivation. The physical and chemical properties of these logs were measured as discussed earlier, before grinding. The 3 replicates were the same, each replicate had 6 experimental growth bags with wood substrates at 6 density categories and 2 bags of control groups of type 1 and type 2. Hence, 18 bags for experiment groups and 6 bags of control groups for 3 of type 1 and 3 of type 2 were used in bag cultivation.

To start the bag cultivation, all the bags were inoculated with 6 cc of mushroom spawn solution through the bottom self-healing injection port. The mushroom spawn culture solution was bought from the Out-Grow company. Because the inoculating solution flowed down and the bottom was lightly saturated, so the bag was shaken to distribute the solution more evenly. After the rye grain layer was 50% colonized with white mycelium (usually 2 – 3 weeks from inoculation), the entire substrate of rye berry and wood waste substrate were mixed in the bags. It took 3 weeks for the further colonization of mycelium. When the full colonization was completed, the fruiting body was expected to appear and develop in the bags after 3 weeks. It was expected that after another 3-4 weeks, the fruiting body would mature and mushrooms could be harvested. However, the growth was stuck before the fruiting body stage.

Table IV-1. Anticipated timetable of the bag cultivation experiment

Activity	Implementation Time			Responsibility
	Week 1	Week 4 - 6	Week 3 - 4	
1. Log physical and chemical properties measurement	xxxxxx			Peiyao
2. The colonize of mycelium in bags		xxxxxx		Peiyao
3. Fruiting body development in bags			xxxxxx	Peiyao
4. Harvest mushroom evaluation			xxxxxx	Peiyao

IV.4 Methodology

IV.4.1 Growth chamber environmental control

The environmental conditions were the same as for the log cultivation experiment. Temperature, relative humidity, air velocity and light intensity were all controlled through the bag cultivation process.

IV.5 Bag cultivation process of wood ear mushrooms

The first replicate started on June 24th (2018) in the left side of the growth chamber. The second replicate was started on July 2nd in the other side. On August 23rd, the third replicate was started in the side with the first batch. During this period, the first batch was ready to start fruiting body development and needed diffused light. But the third replicate just started incubation which preferred a darker environment. So an aluminum foil cover was used to prevent light from affecting the spawn incubation of the third group.

The incubation period was expected to be 4 - 6 weeks for the 100% colonization of mycelium. And it was estimated to take 3 - 4 weeks for fruiting body development. Hence, the total growth cycle for wood ear mushrooms was 7 - 10 weeks. The growth bags were put inside the chamber under the required environmental conditions as discussed earlier.

IV.5.1 Experimental group growth process of period over different category

Figure IV-3 showed the bags just after the liquid spore injection. The spore solution was distributed evenly in the small bags (4 cm × 7.62 cm × 45.72 cm). There was only rye berry substrate inside bags during spore incubation.

Figure IV-3. Bags just after spore inoculation

Figure IV-4 showed the growth bags after one week of spore inoculation. The white mycelium had appeared in some bags.

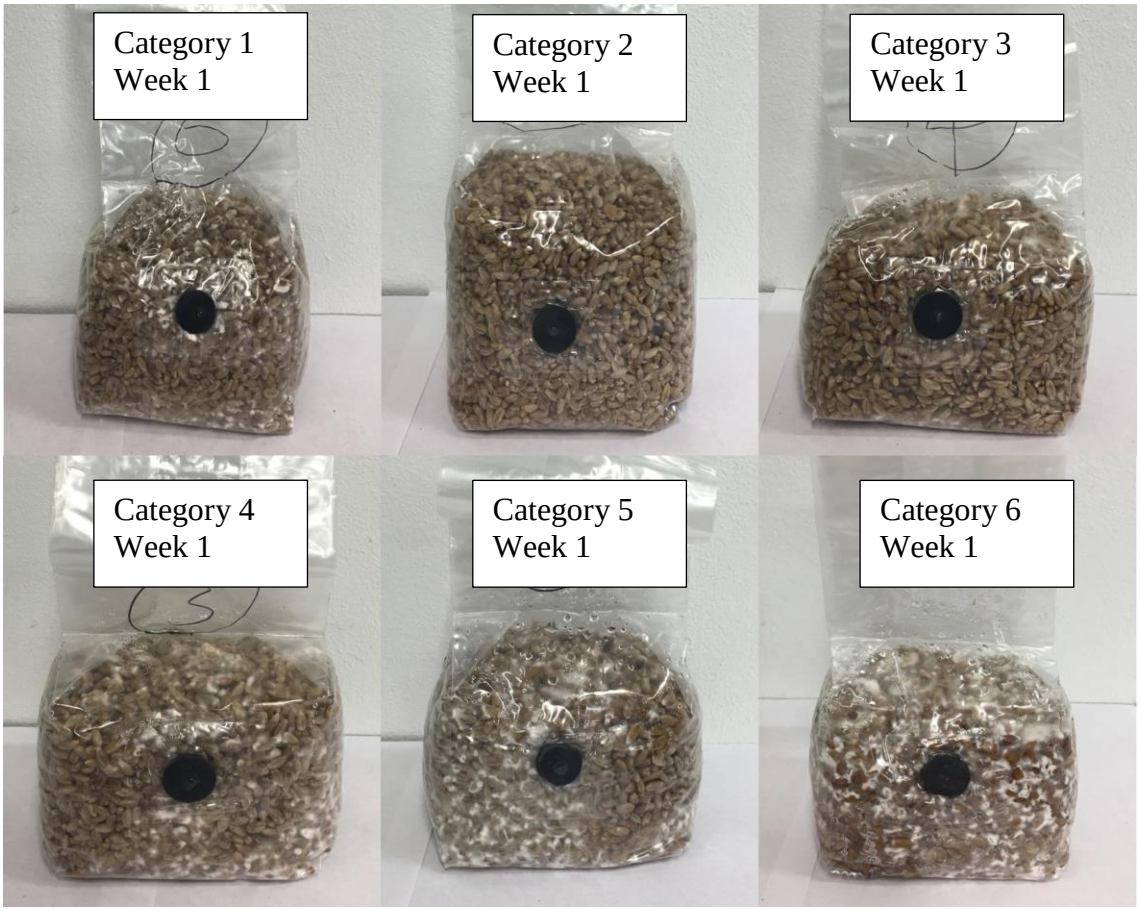


Figure IV-4. Growth bags after one week of inoculation

After two weeks of the inoculation, it seemed that more white mycelium appeared inside the bags, Figure IV-5. It still needed more time for complete colonization of mycelium.

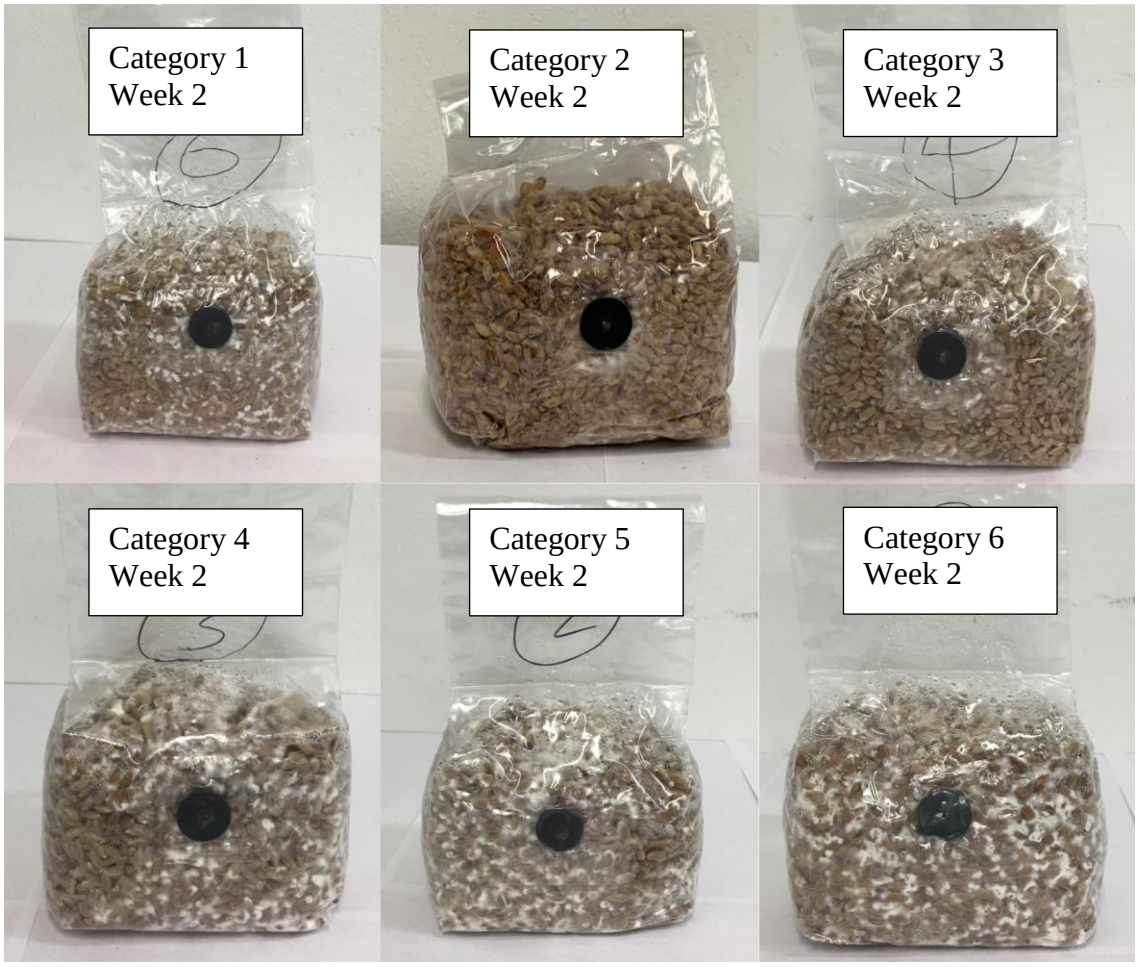


Figure IV-5. Growth bags after 2 weeks of inoculation

For 3 weeks after inoculation, mycelium appeared everywhere in the bags. It could be estimated that almost 40% of each bag was colonized, Figure IV-6. One week was needed for further colonization.

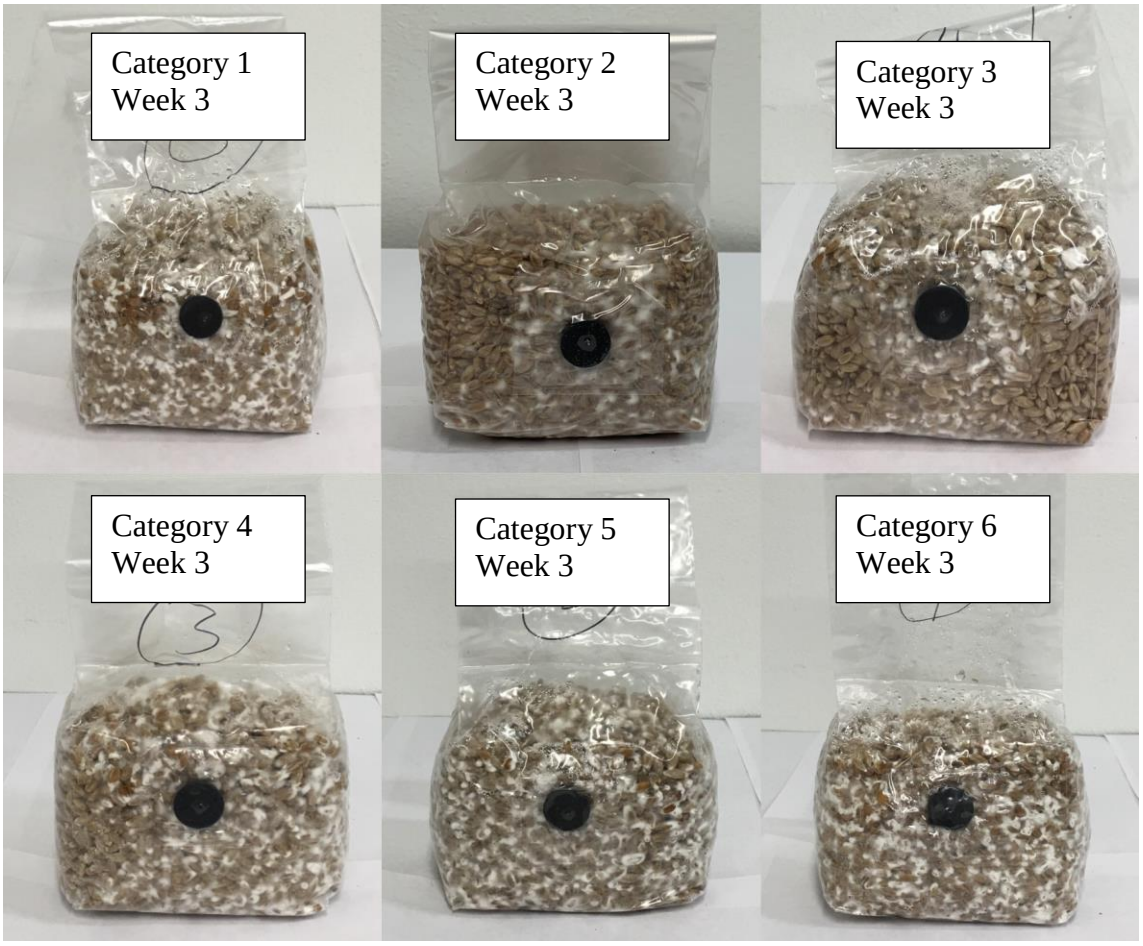


Figure IV-6. Growth bags after 3 weeks of inoculation and then mixed with wood waste immediately at beginning of week 4

One more week for mycelium growth, the bags were ready to add the wood waste substrate for later fruiting body development in week 4. The wood waste substrate was made from the pecan logs. These logs were also selected from the 104 logs. For each density category, logs with bigger diameter and closer to the visual standards were chosen to make the wood substrate. Since logs had been divided into 6 categories by densities, wood substrates were also divided into 6 categories based on the density of the intact logs. Logs were chipped by a chipper shredder (Portland, 90293) to small pieces, then ground by a wood grinder (CHENGDA, CF158) to smaller size wood waste with size less than 0.2 *cm*. The chipper was a Portland chipper shredder whose maximum chipping diameter of logs was 3.8 *cm* (1-1/2 in.). Logs were cut into smaller pieces to fit the chipper. The small log pieces were then ground by Wood grinder to much smaller chips. The maximum grinding size was 2.54 *cm* (1in.) of wood diameter for this wood grinder.

Figure IV-7. Chipper shredder (Portland, 90293) and Wood grinder (CHENGDA, CF158)

The rye berry and colonized mycelium in the 6 small bags as shown in the previous figures were transferred to bigger bags (12.7 cm × 10.16 cm × 45.72 cm). Then wood waste substrates were added into these bags. Everything was mixed evenly in the big bags. Since wood waste was made by logs at 6 different density categories, the bags were also defined according to the 6 categories.

Figure IV-8 showed the 6 big bags at 6 categories after one week of substrate mixing in week 5. The numbers in the figure represented the categories of the bag substrate.

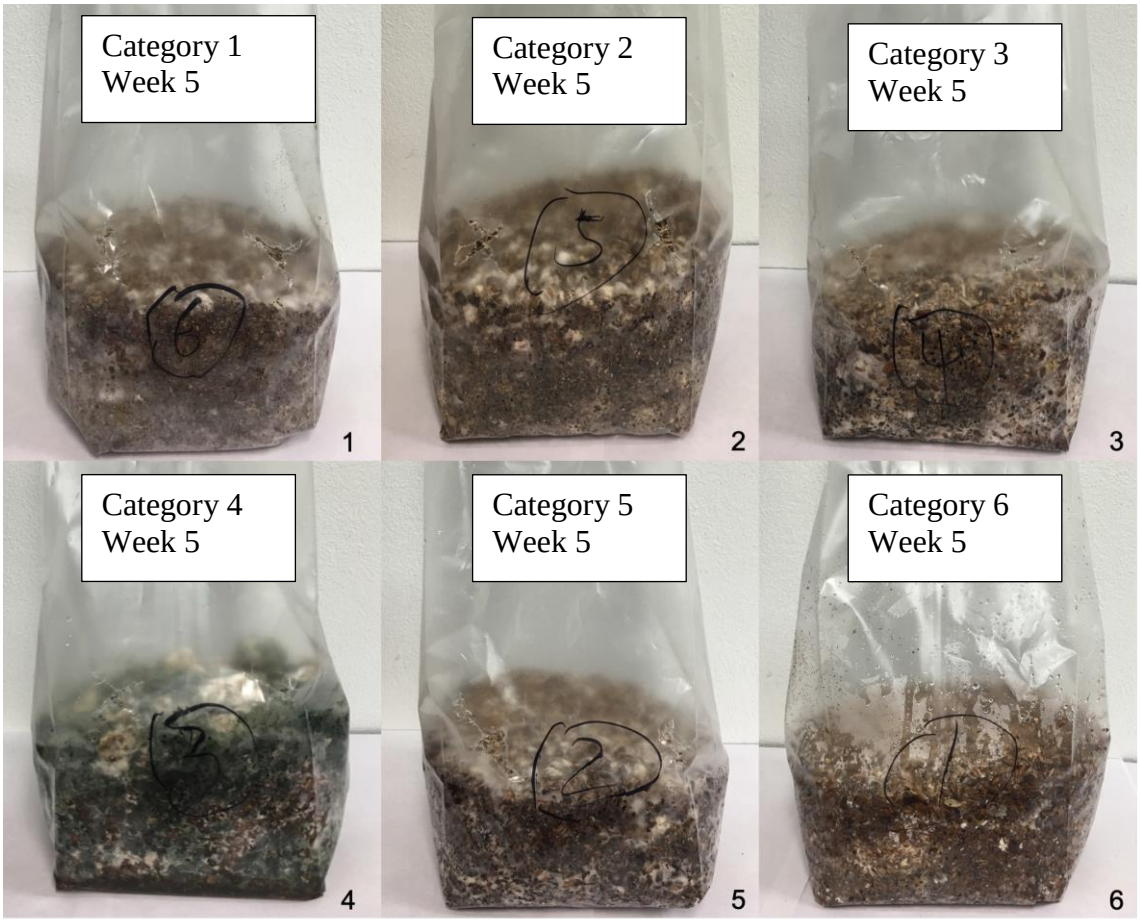


Figure IV-8. 5 weeks after inoculation and 1 week after mixing with wood waste substrate

After two weeks for the further mycelium growth in the big bags, it was almost fully colonized for all bags. The top parts of the bags were cut off to prepare for fruiting body development in the first replicate. This period was called fruiting body development stage. During this period, water was added and high relative humidity was provided. RO water was added twice a day. The light was on blue and red spectrum and was set at 500 lux intensity for fruiting body growth. Small holes were made under the bags for water drainage. Figure IV-9 showed these 6 bags of the first replicate over different categories with the front and top view. Mycelium still grew and bags with low density categories had better growth conditions.

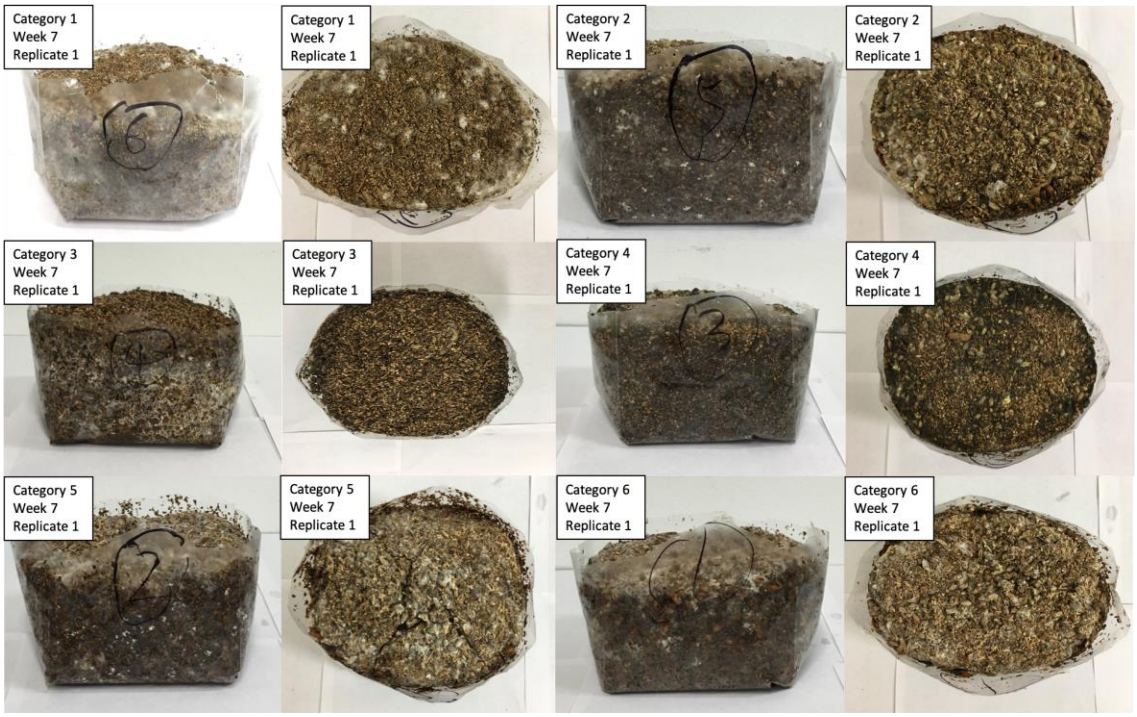


Figure IV-9. 7 weeks after inoculation and 3 weeks after substrate mixing then cut the top off immediately

9 weeks after inoculation, it seemed that there were still white mycelium and no fruiting body appeared during the fruiting body development stage, Figure IV-10. Bags continued to receive water and relative humidity in the growth chamber was controlled above 70 %. The density categories of mycelium growth were ranked from the most to least and was: 1 - 2 - 3 - 4 - 6 - 5. This ranking was done by visual observation.

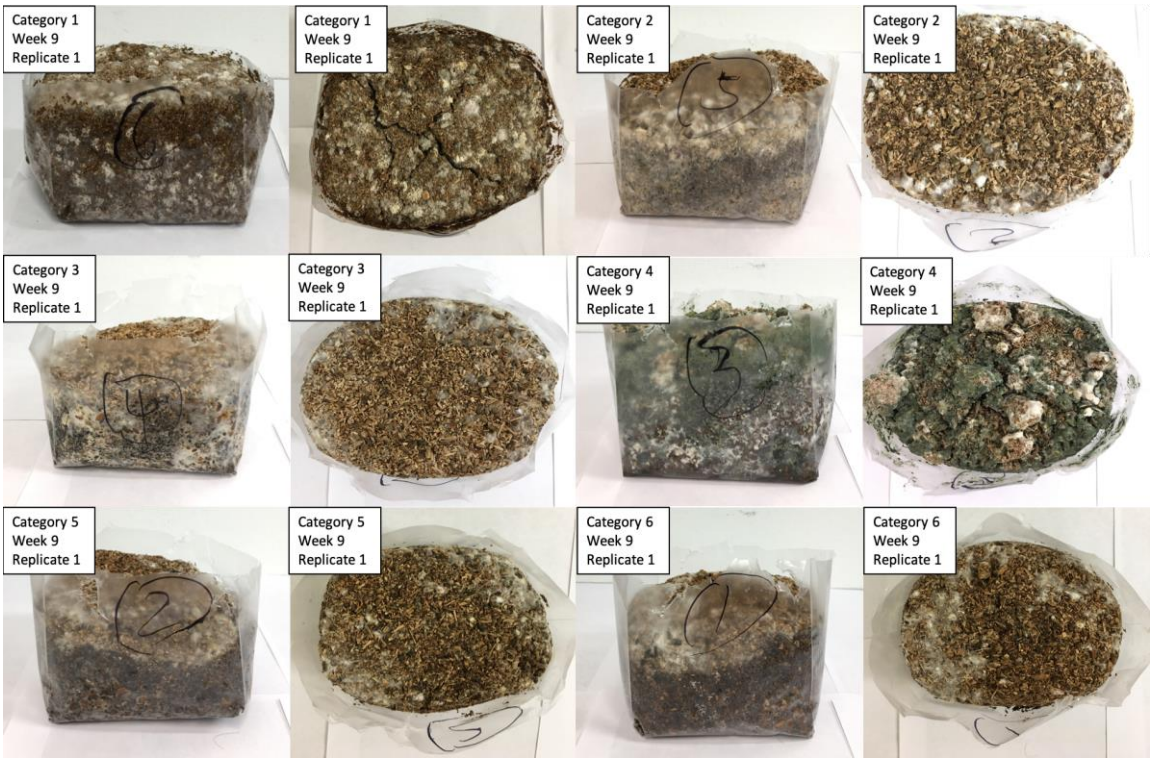


Figure IV-10. 9 weeks after inoculation and 5 weeks after mixing

For 11 weeks after inoculation, there was no obvious sign for fruiting body appearance, Figure IV-11. At this time, mycelium died and only a few were left. Compared to the wood waste substrate used in this research and other substrate used by other farmers, it may be the shortage of nutrition.

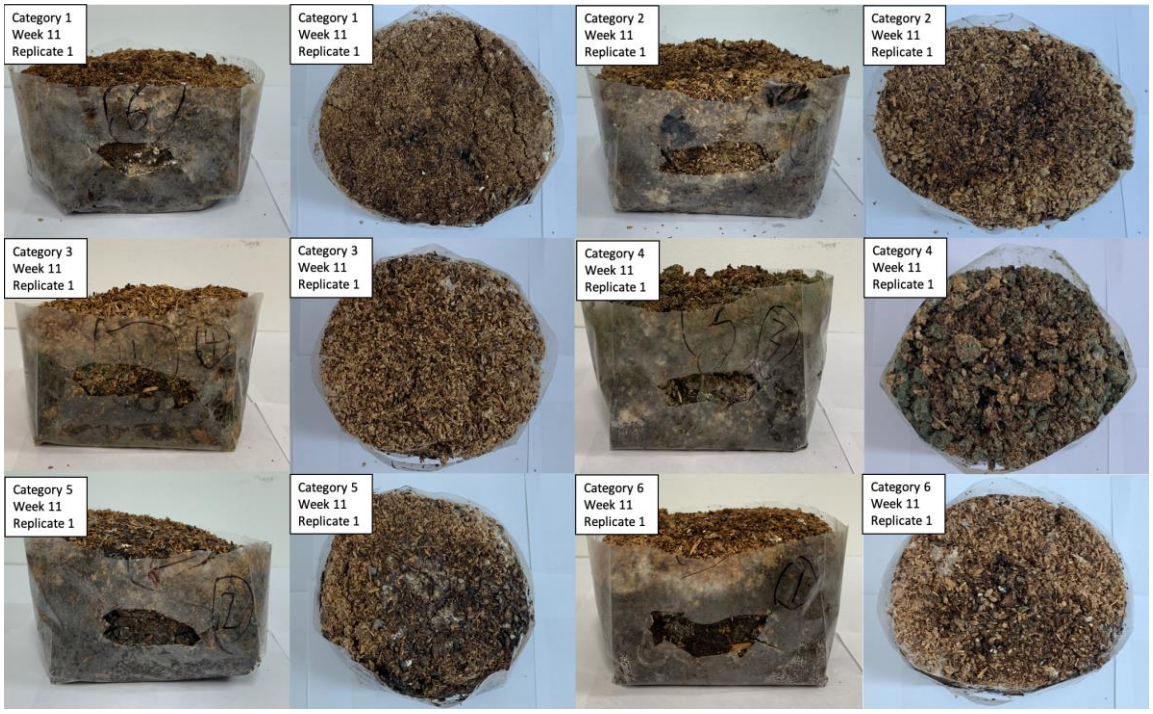


Figure IV-11. 11 weeks after inoculation and 7 weeks after substrate mixing

Figure IV-12 showed the second replicate after cutting the top for fruiting body growth in week 7. The timeline was same as replicate 1. Mycelium grew and no fruiting body appeared. The environmental conditions were kept the same as the first replicate.

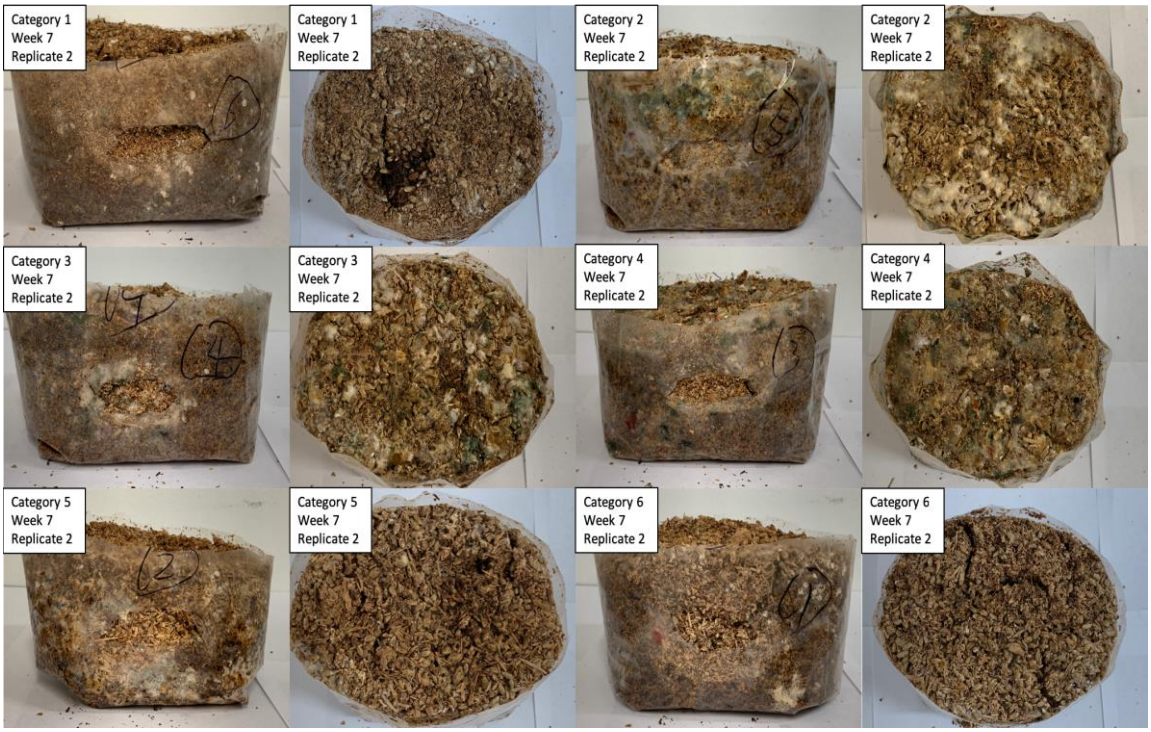


Figure IV-12. Second replicate: 7 weeks after inoculation and 3 weeks after mixing then cut the top off immediately

Two more weeks after cutting the top, there was no fruiting body; however, mycelium still grew. During week 9, mycelium reached highest growth level. Ranking mycelium growth from the most to the least yields: 2 - 3 - 4 - 1 - 5 - 6.

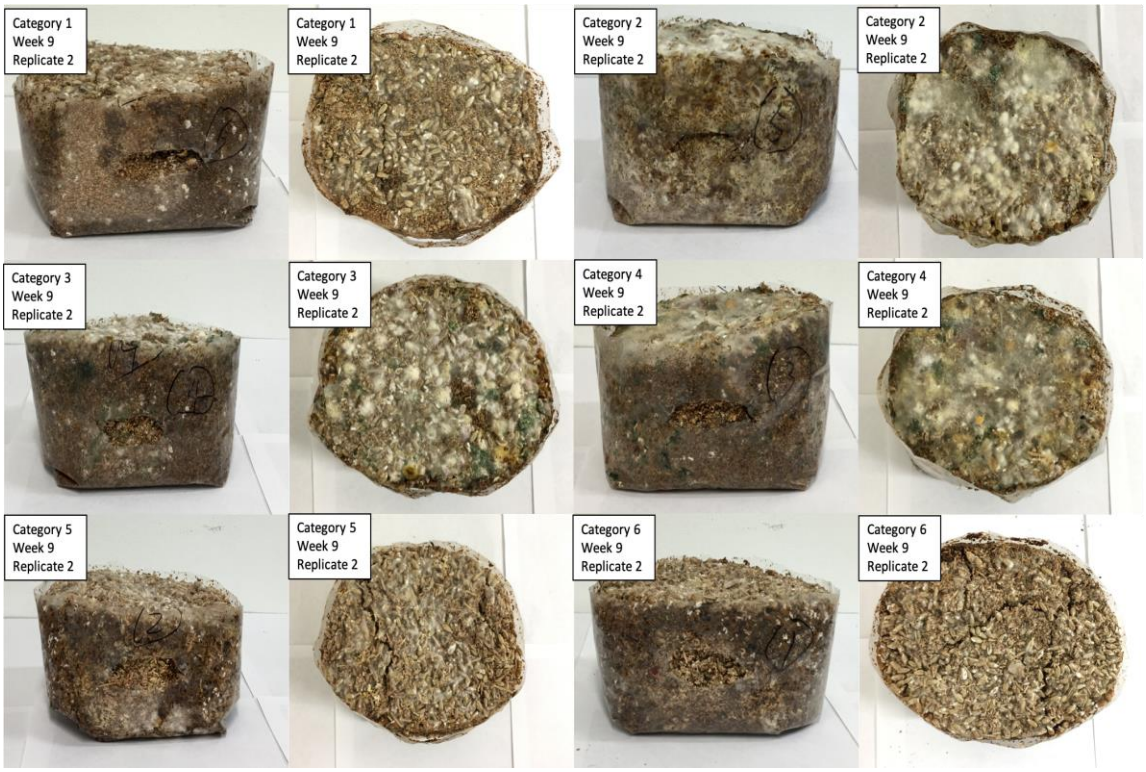


Figure IV-13. Second replicate: 9 weeks after inoculation and 5 weeks after mixing

After it reached its maximum growth, mycelium started to decrease and died later. However, there was still no fruiting body appearance. Only bags of category 1, 2, 5 and 6 still had some mycelium left.

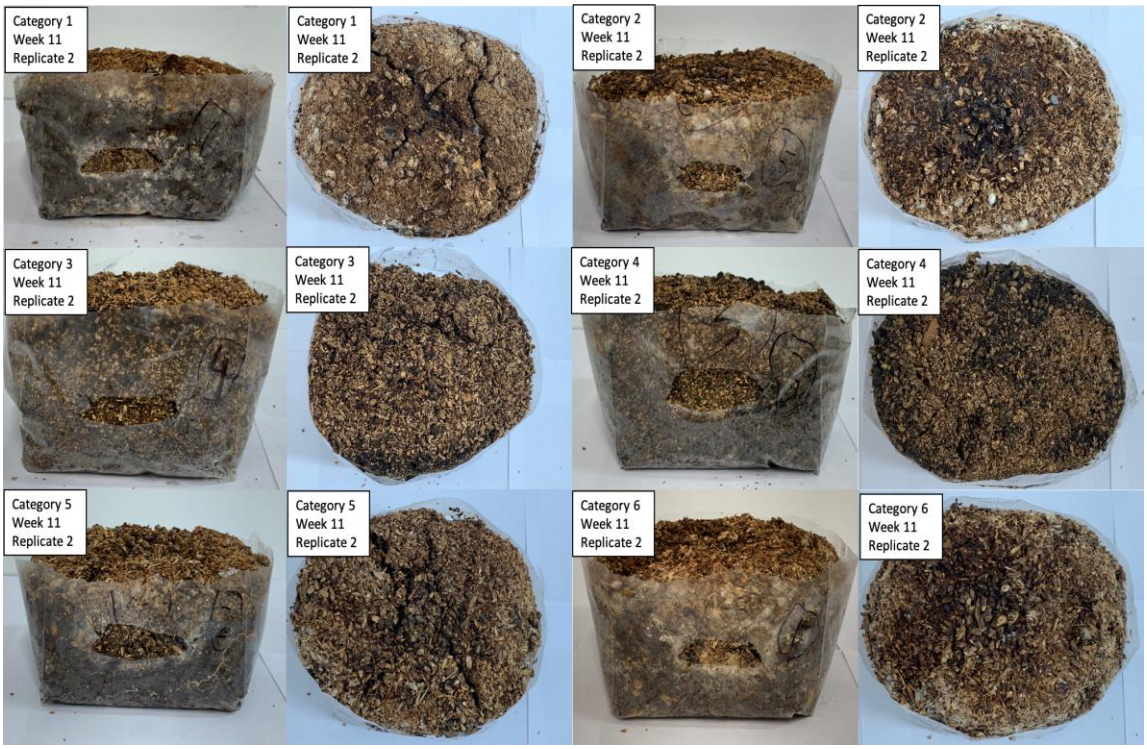


Figure IV-14. Second replicate: 11 weeks after inoculation and 7 weeks after substrate mixing

Figure IV-15 was the third replicate of the bags after substrates mixing. In here, the top of the bags was not cut anymore to prevent moisture from running out too fast. Mycelium still grew and fruiting body stage was not ready.

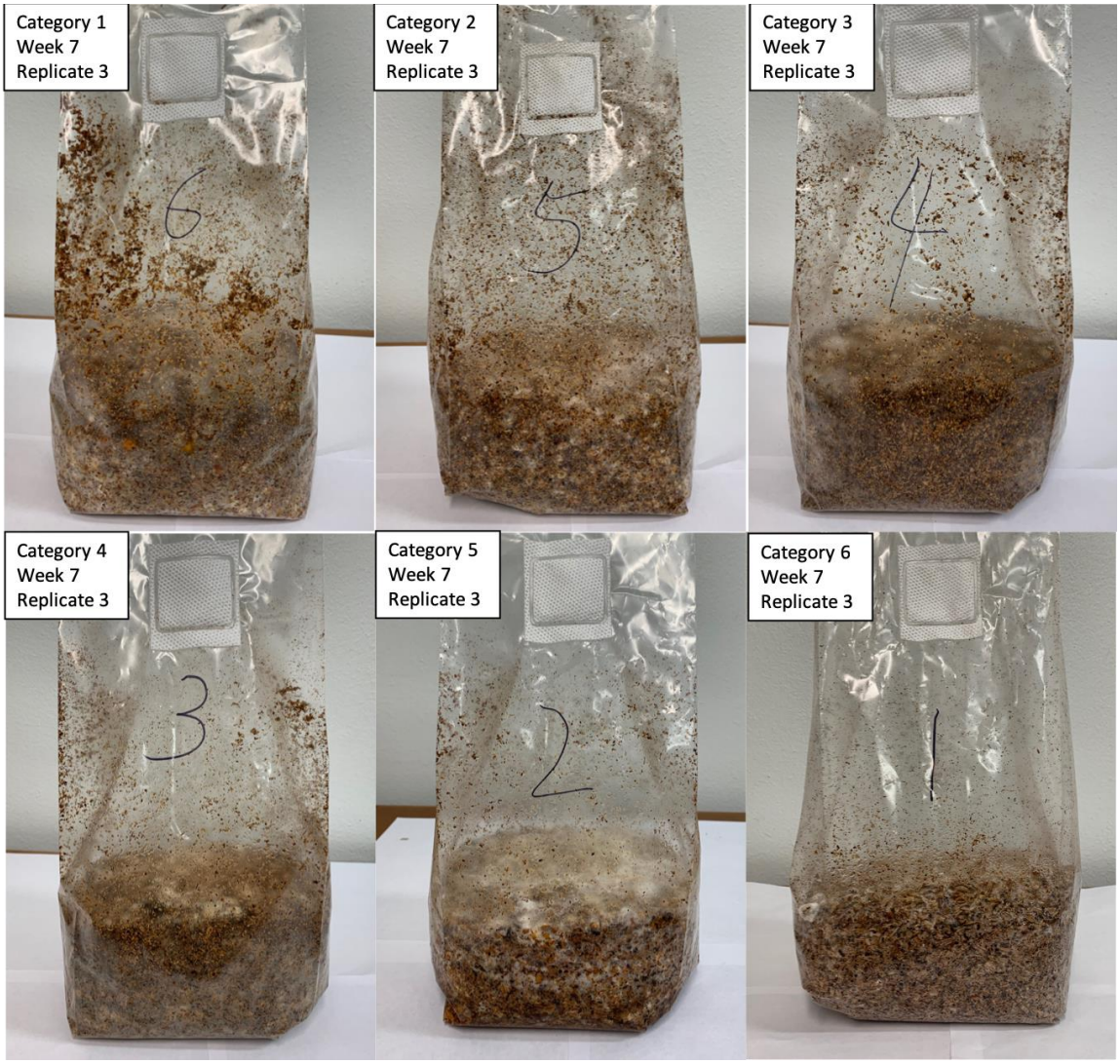


Figure IV-15. Third replicate: 7 weeks after inoculation and 3 weeks after mixing

After two more weeks for mycelium growth, some X cuts were made in the bags on the front and bottom sides, Figure IV-16. Some mycelium started to die instantly. Some died in the bags (category 2, 3 and 5), but new mycelium appeared in the bag of category 6. Bags were watered through these cuts every day.

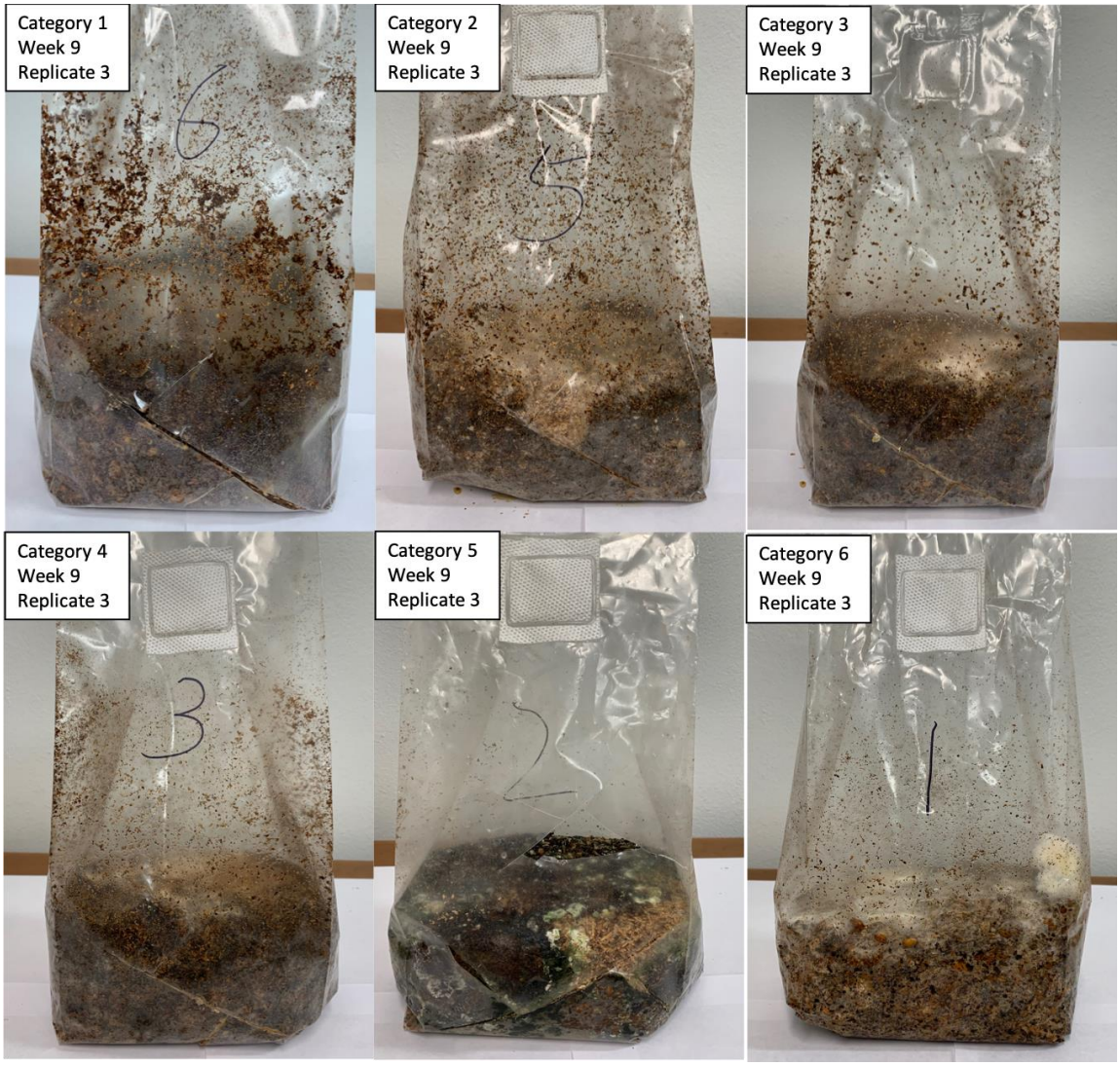


Figure IV-16. Third replicate: 9 weeks after inoculation and 5 weeks after mixing

In week 11, mycelium continued to decrease. No fruiting body appeared. Bags of category 2, 3 and 5 had some mycelium left. In bag of category 5, some mycelium turned to black.

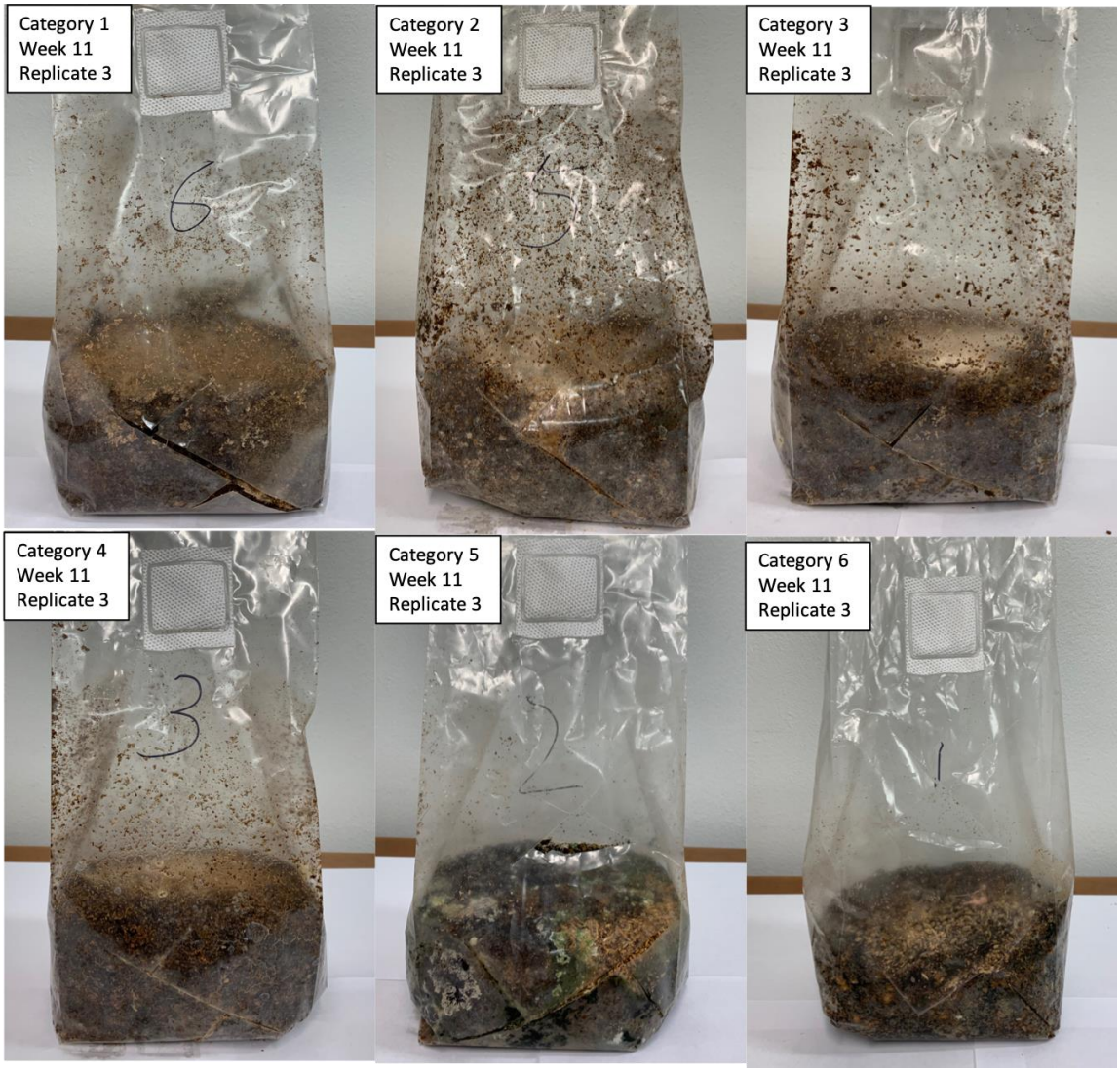


Figure IV-17. Third replicate: 11 weeks after inoculation and 7 weeks after mixing

For another two weeks, there was only a few mycelium left. And fruiting body didn't appear yet.

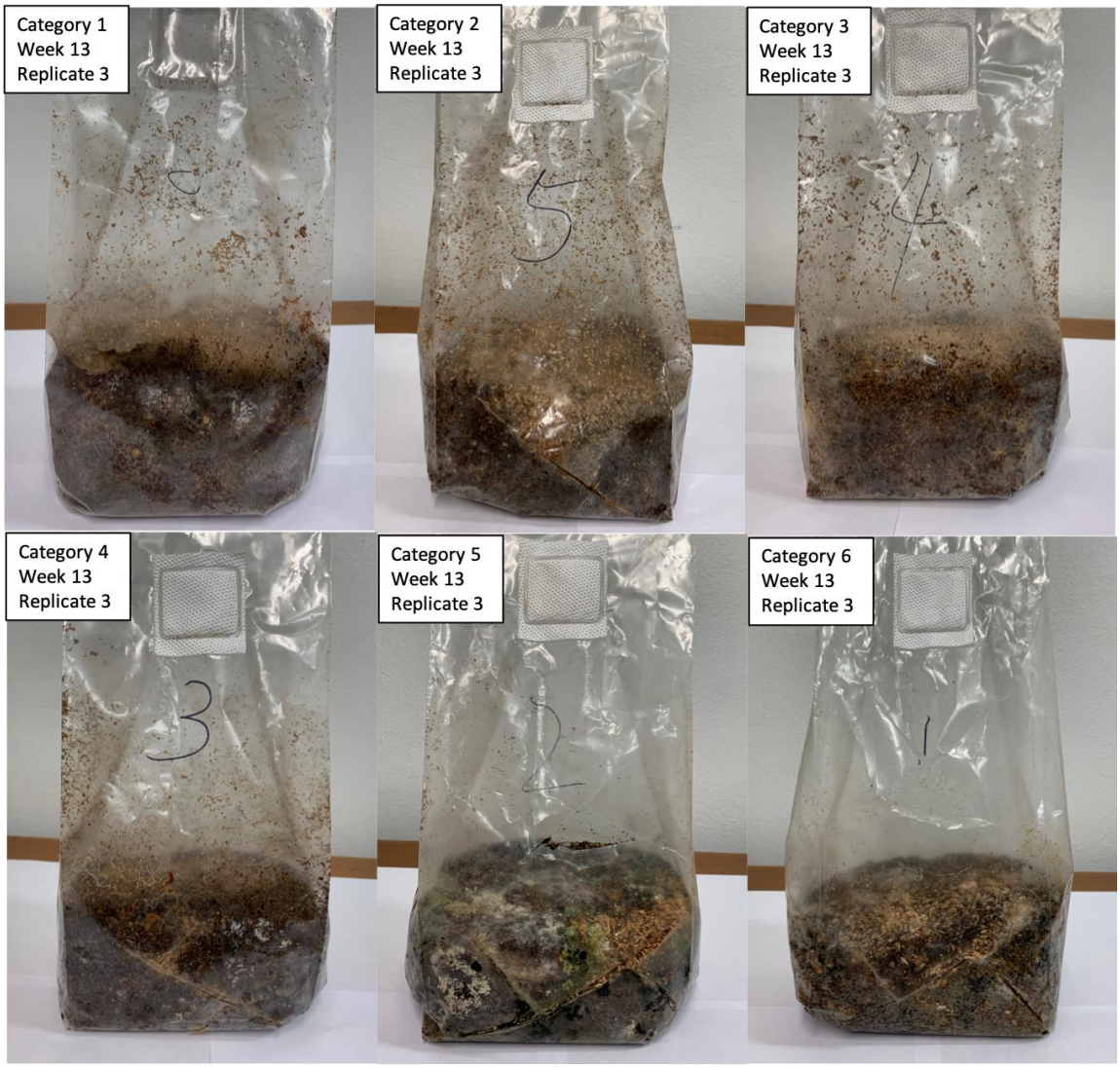


Figure IV-18. Third replicate: 13 weeks after inoculation and 9 weeks after mixing

IV.5.2 Bags in the same category over time period

In here, bags in the same category were compared over time. Figure IV-19 showed the growth process of the bags in category 1. Week 0 indicated the bag just after spore inoculation. During the beginning of week 4, the substrate in bag was transferred to big bags and wood waste substrate was added immediately. In week 5, more mycelium appeared in the bag after one more week of mycelium growth in mixed substrates.

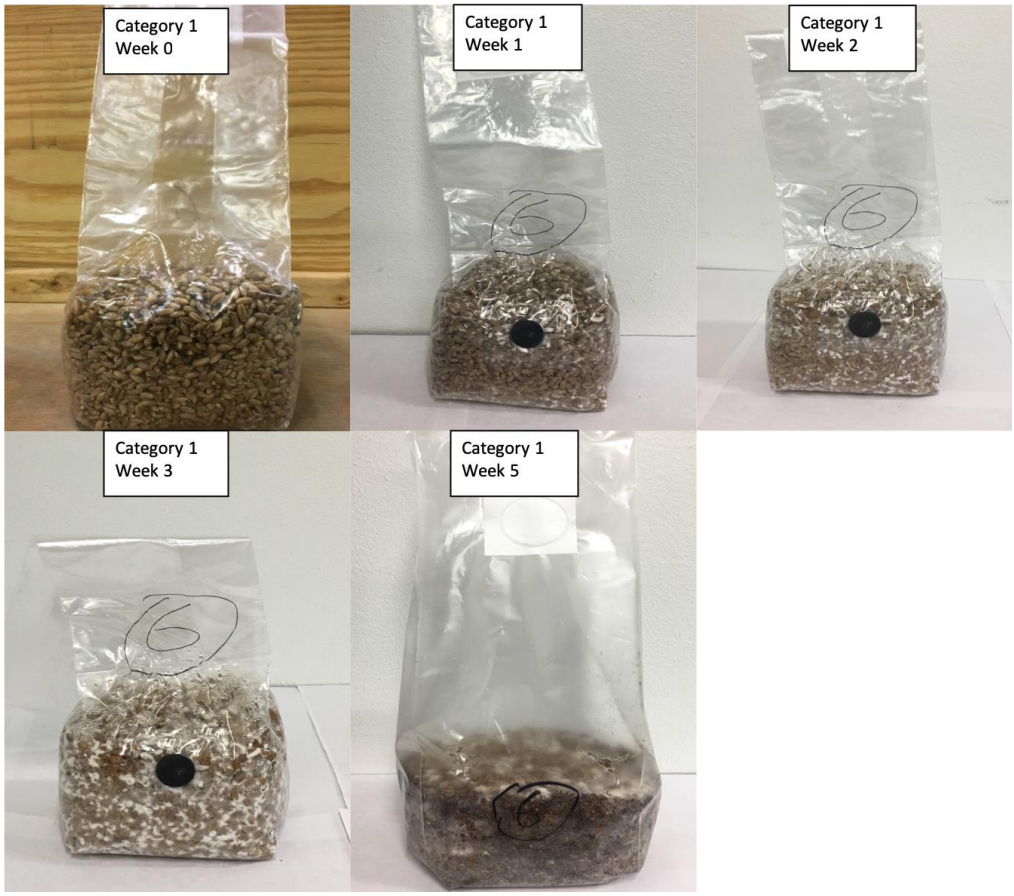


Figure IV-19. Bags of category 1 growth process over time - wood substrate was added at the beginning of week 4

Figure IV -20 to IV-22 showed two more weeks for mycelium growth after week 5. The top of the bag was cut in the beginning of week 7 for replicates 1 and 2; however, X's were cut in the bags of replicate 3. The fruiting body development stage was started. The bags were watered every day to keep it moist. In replicate 3, water was added through these cuts. The mycelium still grew in week 7 and week 9. But after week 11, mycelium decreased and fruiting body didn't appear. There were only a few mycelium left and no sign of fruiting body later.

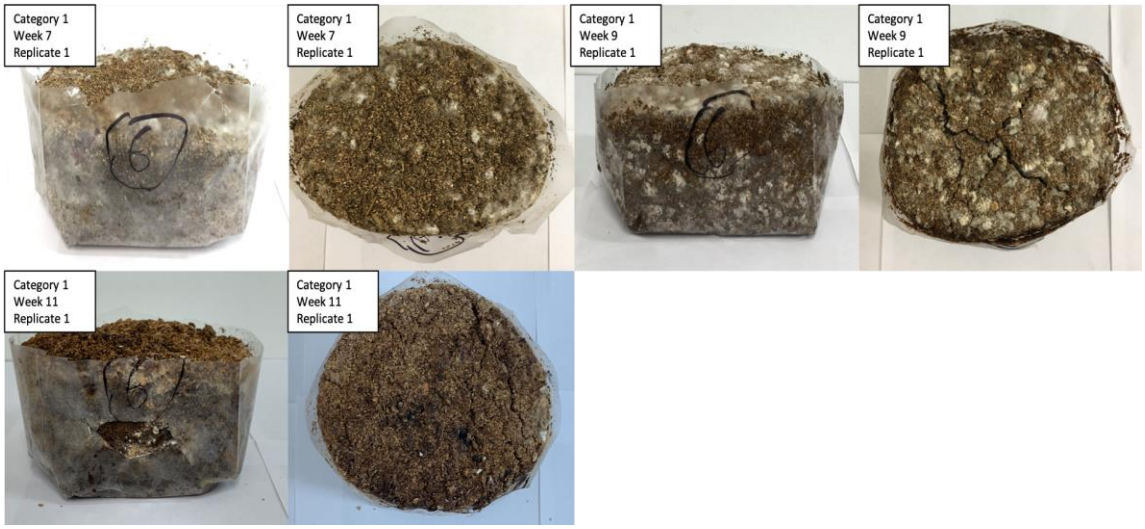


Figure IV-20. Growth bags of replicate 1 of category 1 after cutting the top off for fruiting body development

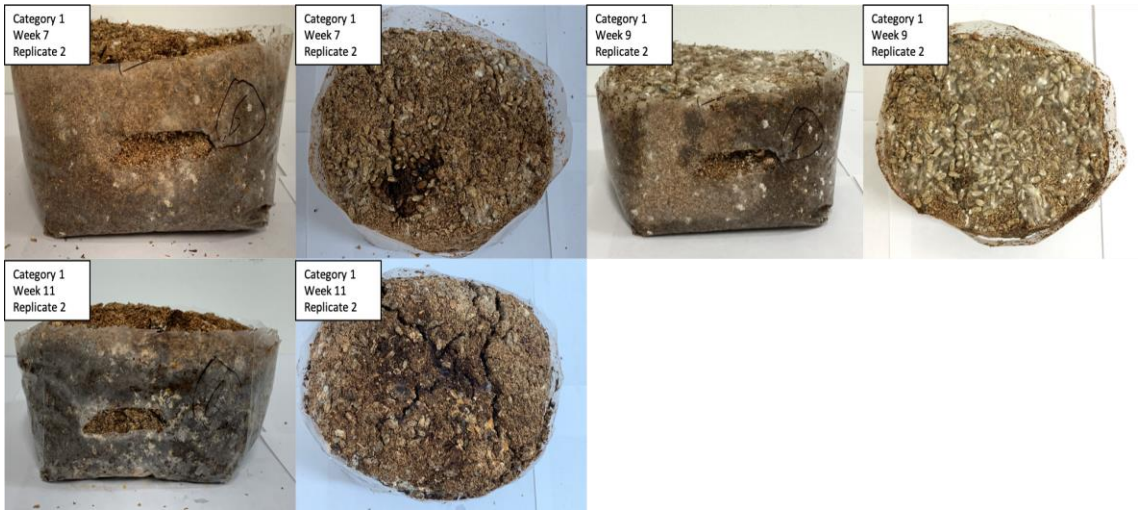


Figure IV-21. Growth bags of replicate 2 of category 1 after cutting the top off for fruiting body development

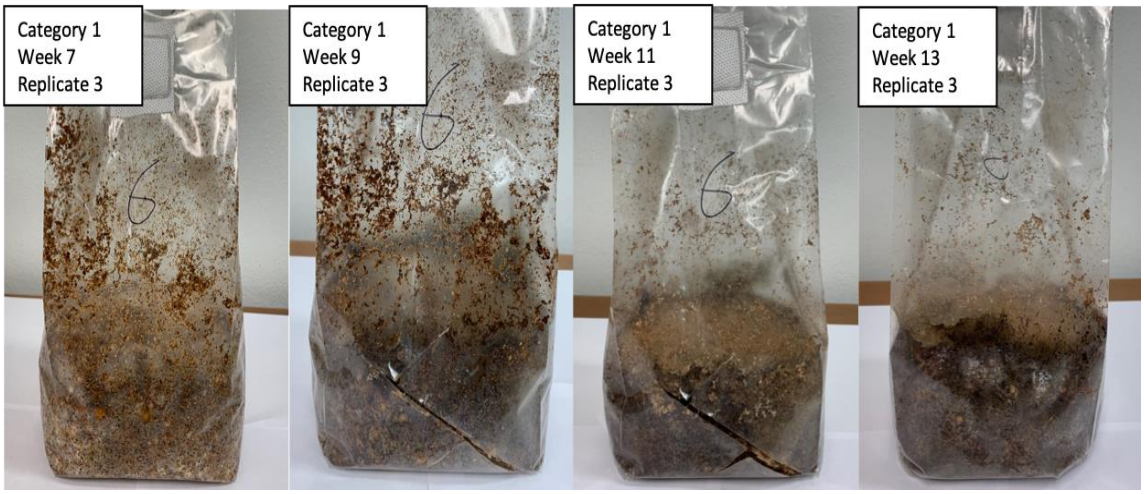


Figure IV-22. Growth bags of replicate 3 of category 1 after X cuts for fruiting body development

In Figure IV-23, shows mycelium growth during week 0 to week 5. The wood waste substrate was also added at the beginning of week 4. Week 5 showed more mycelium appeared after one week from substrates mixing.

Figure IV-23. Bags of category 2 growth process - wood waste was added at the beginning of week 4

For all 3 replicates, the mycelium all reached its maximum growth during week 9, Figure IV- 24 to IV- 26. And mycelium decreased later and only a few were left later. The fruiting body didn't appear.

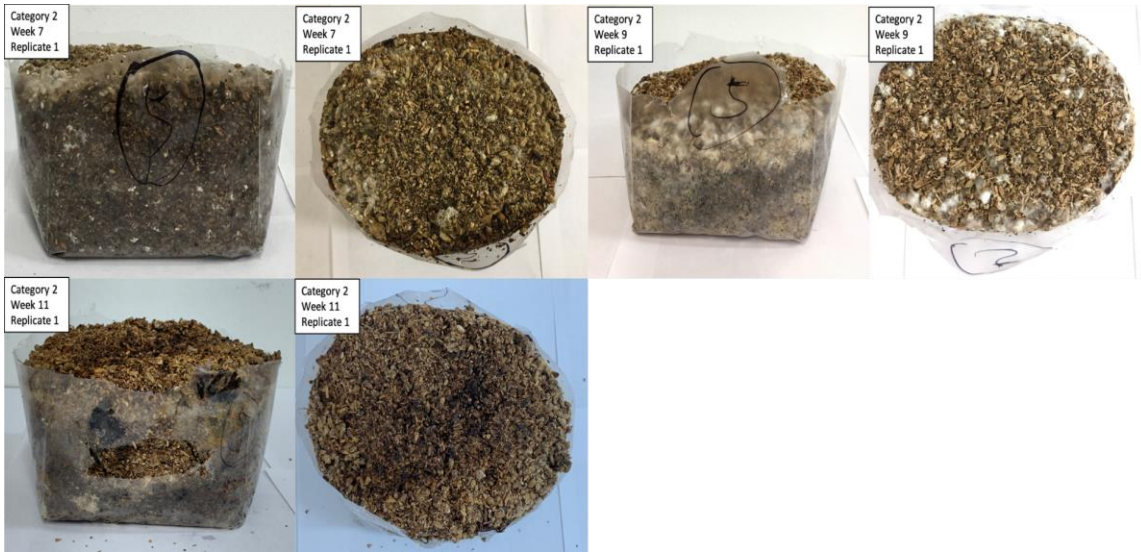


Figure IV-24. Growth bags of replicate 1 of category 2 after cutting top for fruiting body development

Figure IV-25. Growth bags of replicate 2 of category 2 after cutting top for fruiting body development

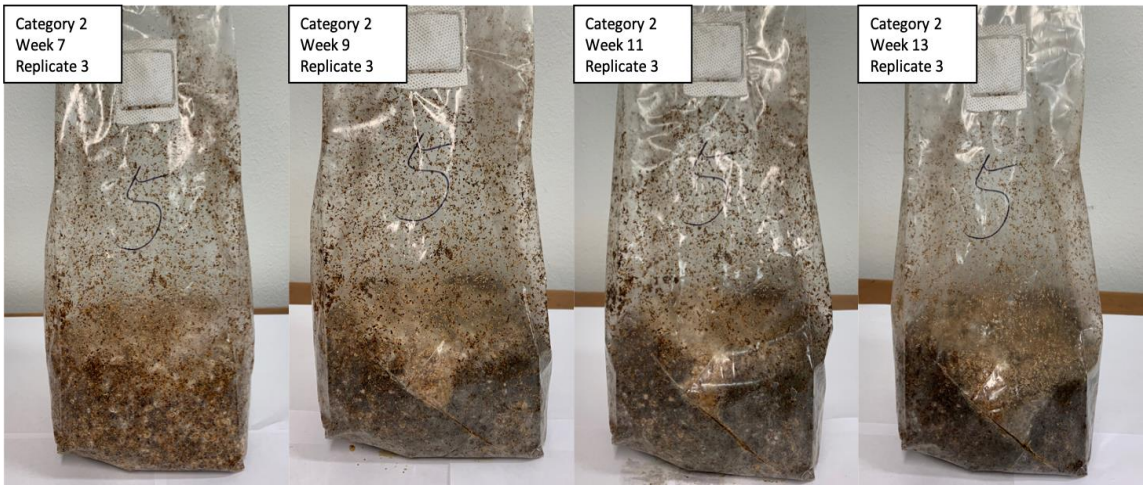


Figure IV-26. Growth bags of replicate 3 of category 2 after X cuts for fruiting body development

There was no difference of mycelium growth in the bags of category 3. The mycelium grew and mixed with wood waste substrate in the beginning of week 4. After one week of mixing, more mycelium appeared in week 5.

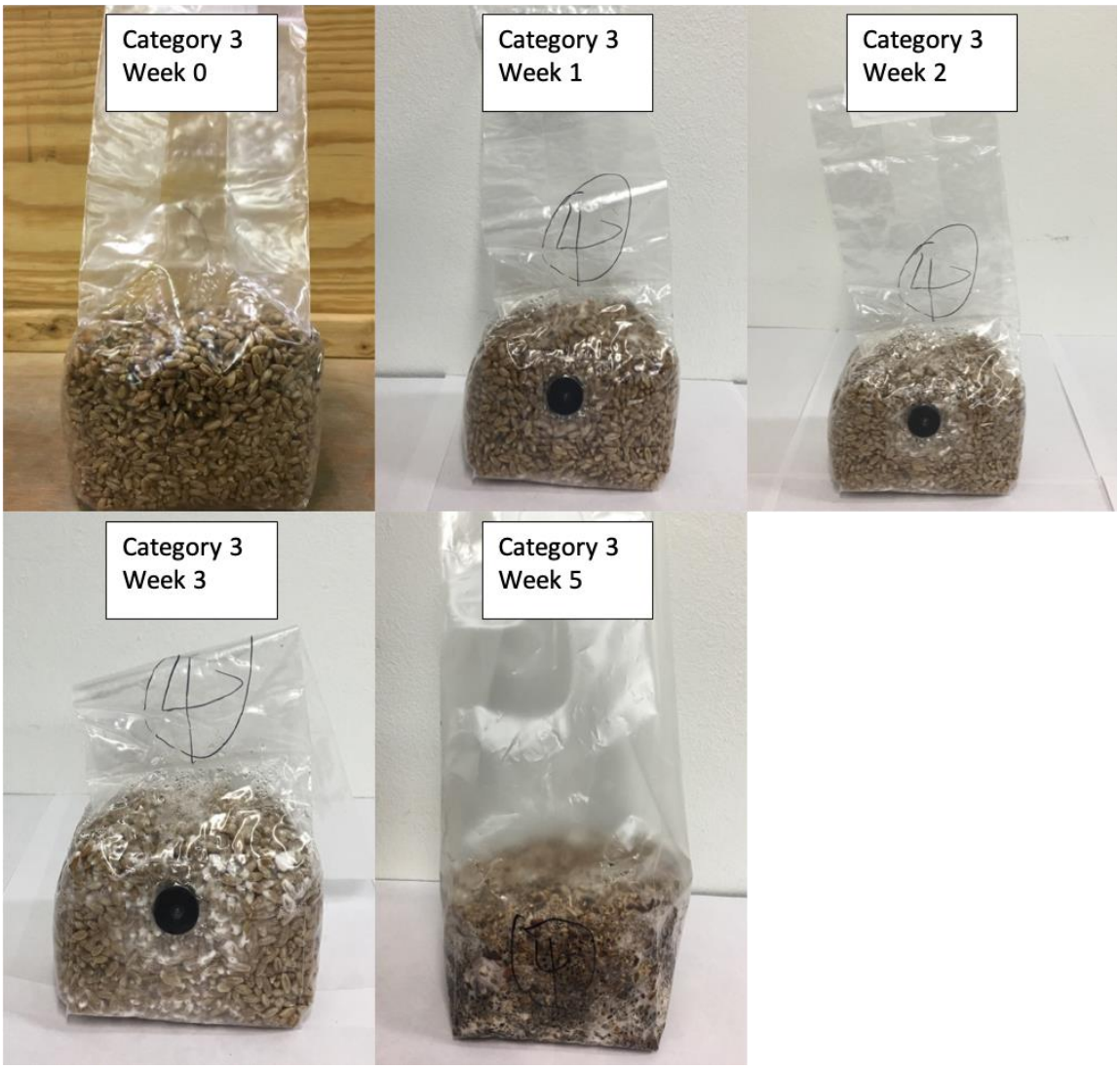


Figure IV-27. Bags of category 3 growth process - wood substrate was added at the beginning of week 4

For category 3, the mycelium also reached growth peak during week 9 for replicates 1 and 2, Figure IV- 28 to IV- 29. But for replicate 3, it was week 7. Later, mycelium of all bags died and disappeared. No fruiting body appeared.

Figure IV-28. Growth bags of replicate 1 of category 3 after cutting top for fruiting body development

Figure IV-29. Growth bags of replicate 2 of category 3 after cutting top for fruiting body development

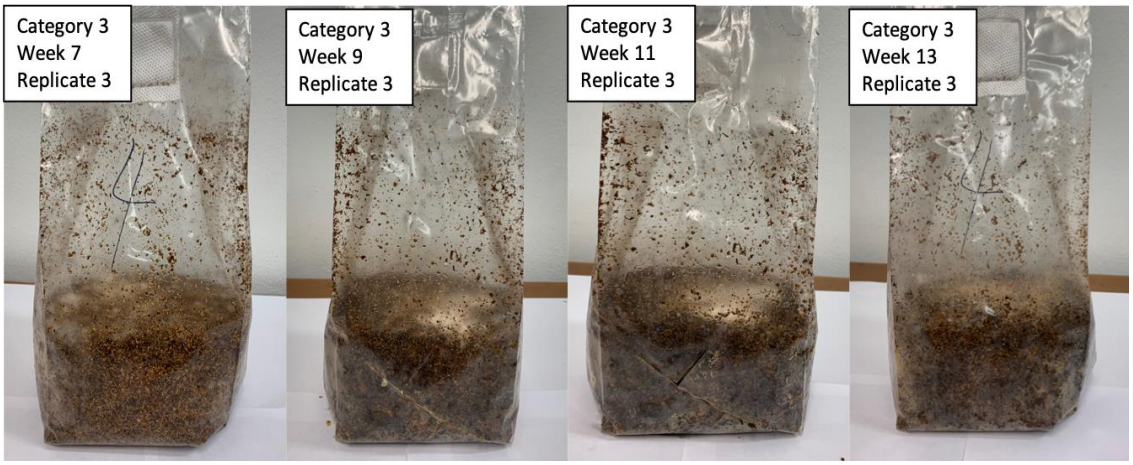


Figure IV-30. Growth bags of replicate 3 of category 3 after X cuts for fruiting body development

In Figure IV-31, mycelium grew during the early 3 weeks and wood waste substrate was added in week 4. One week after mixing, more mycelium appeared in week 5.

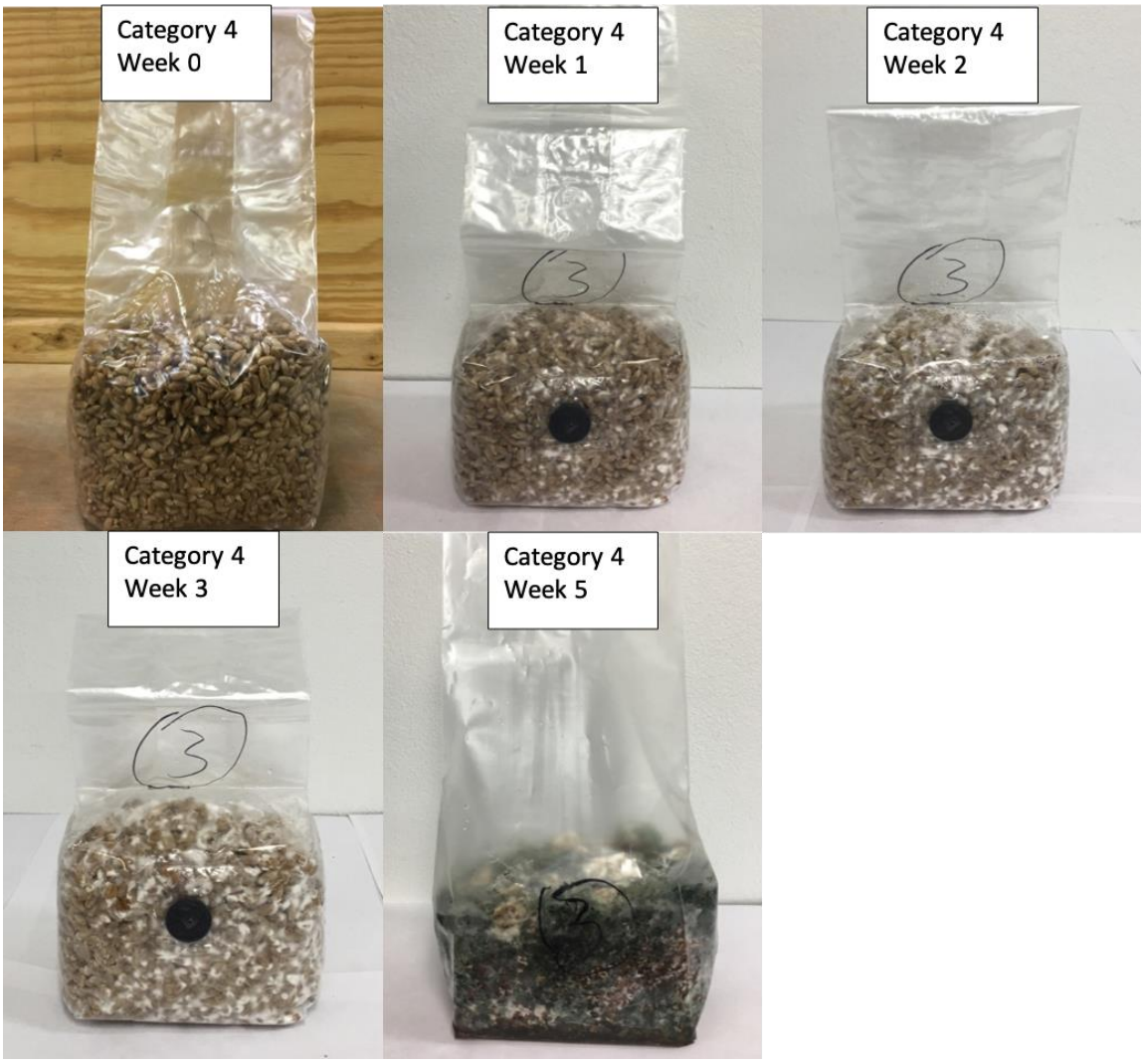


Figure IV-31. Bags of category 4 growth process - wood waste added at beginning of week 4

Mycelium reached the best growth in week 9 for replicates 1 and 2. Mycelium growth was best in week 7 for replicate 3. Later mycelium in all bags decreased. Fruiting body didn't appear.

Figure IV-32. Growth bags of replicate 1 of category 4 after cutting top for fruiting body development

Figure IV-33. Growth bags of replicate 2 of category 4 after cutting top for fruiting body development

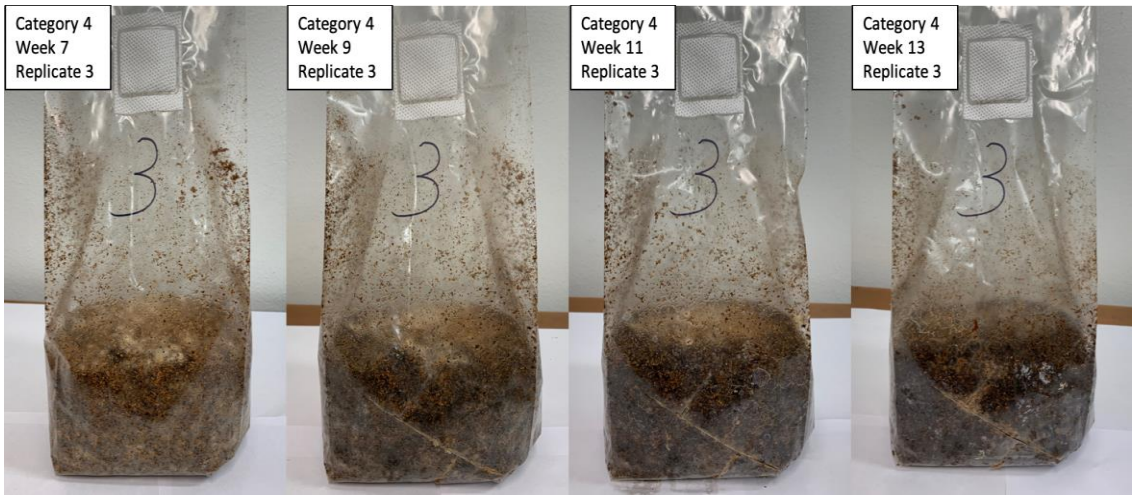


Figure IV-34. Growth bags of replicate 3 of category 4 after X cuts for fruiting body development

In Figure IV-35, mycelium grew and wood substrate was added in week 4. In week 5, mycelium grew in mixed substrates.

Figure IV-35. Bags of category 5 growth process - wood substrate added at beginning of week 4

For the fruiting body development stage, mycelium still grew and reached the maximum amount in week 9 for replicates 1 and 2, Figure IV-36 to IV-37. But for replicate 3, mycelium growth reached its highest level in week 7, Figure IV-38. However, fruiting body didn't appear and mycelium died later for all replicates.

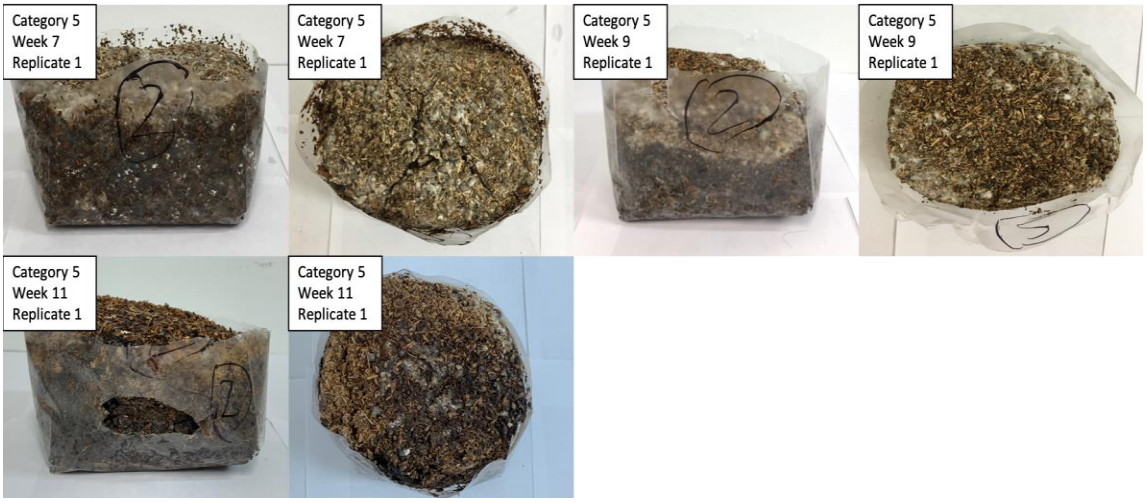


Figure IV-36. Growth bags of replicate 1 of category 5 after cutting top for fruiting body development

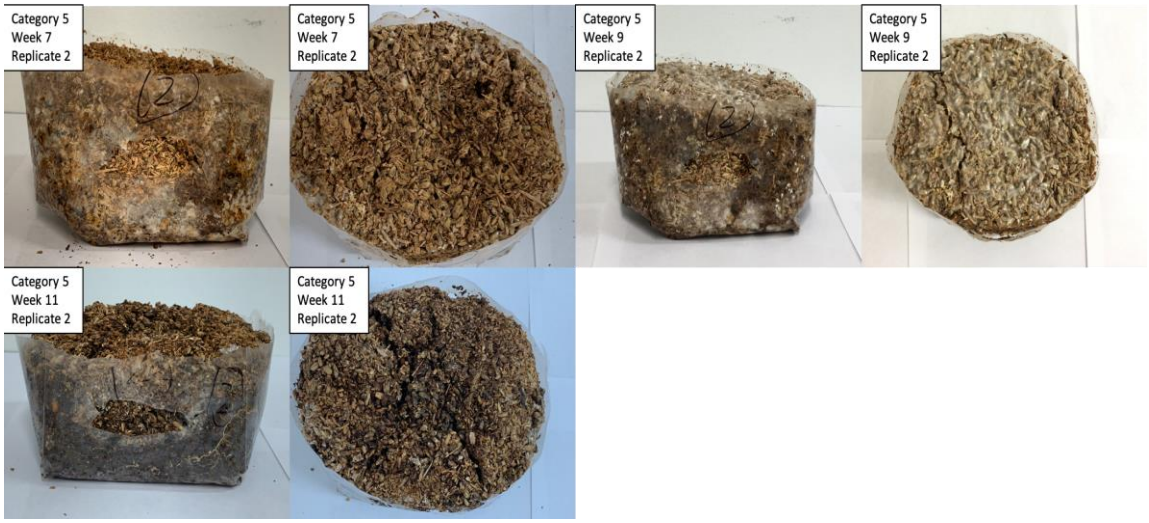


Figure IV-37. Growth bags of replicate 2 of category 5 after cutting top for fruiting body development

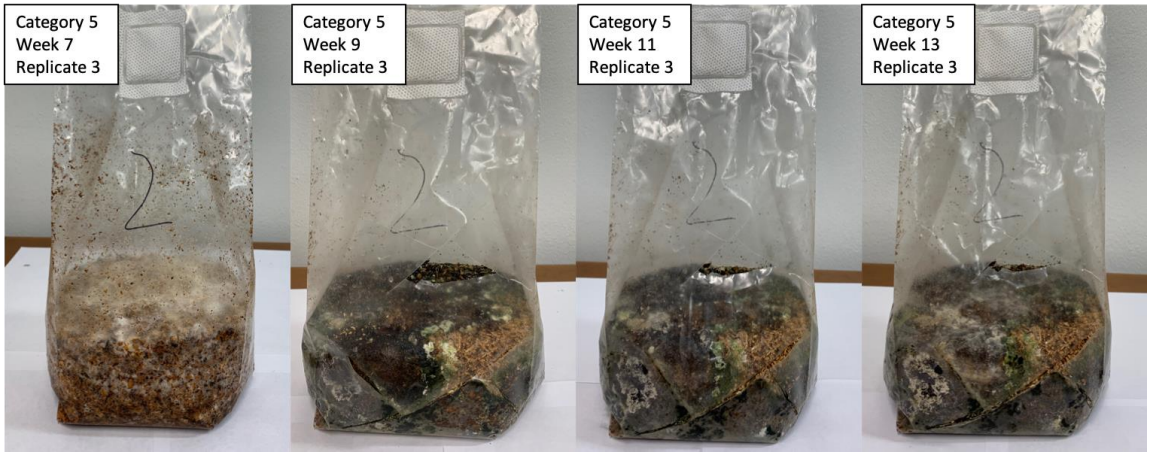


Figure IV-38. Growth bags of replicate 3 of category 5 after X cuts for fruiting body development

For the early mycelium growth period, it was still the same and mycelium grew from week 0 to week 5. And rye berry was mixed with wood waste in week 4, Figure IV-39.

Figure IV-39. Bags of category 1 growth process - wood substrate added at beginning of week 4

After cutting top in week 7 for replicates 1 and 2, it was time for fruiting body growth. For replicate 3, X cuts were made for fruiting body stage. Mycelium still grew, but no sign of fruiting body appearance.

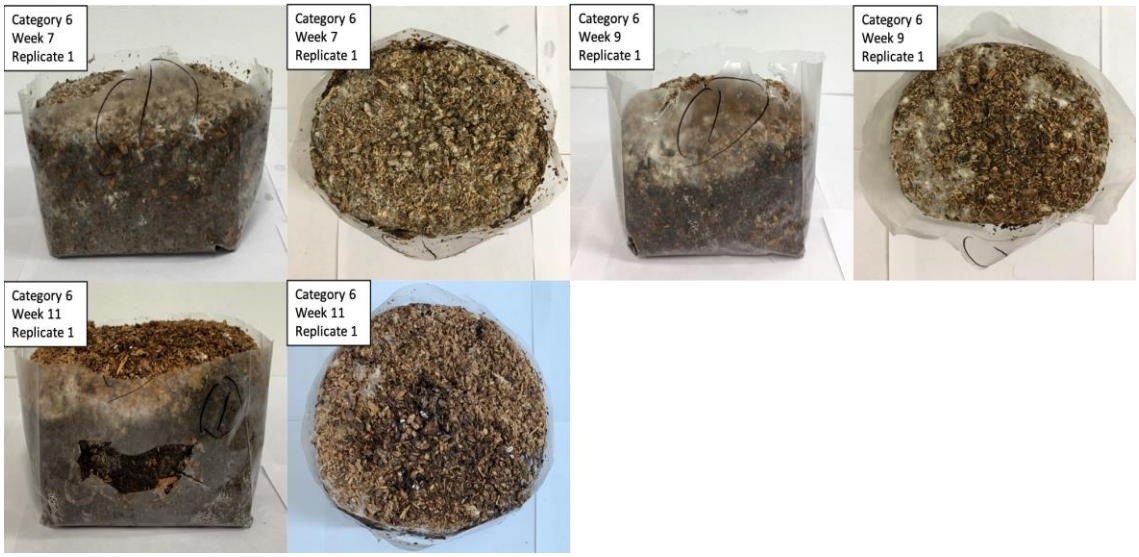


Figure IV-40. Growth bags of replicate 1 of category 6 after cutting top for fruiting body development

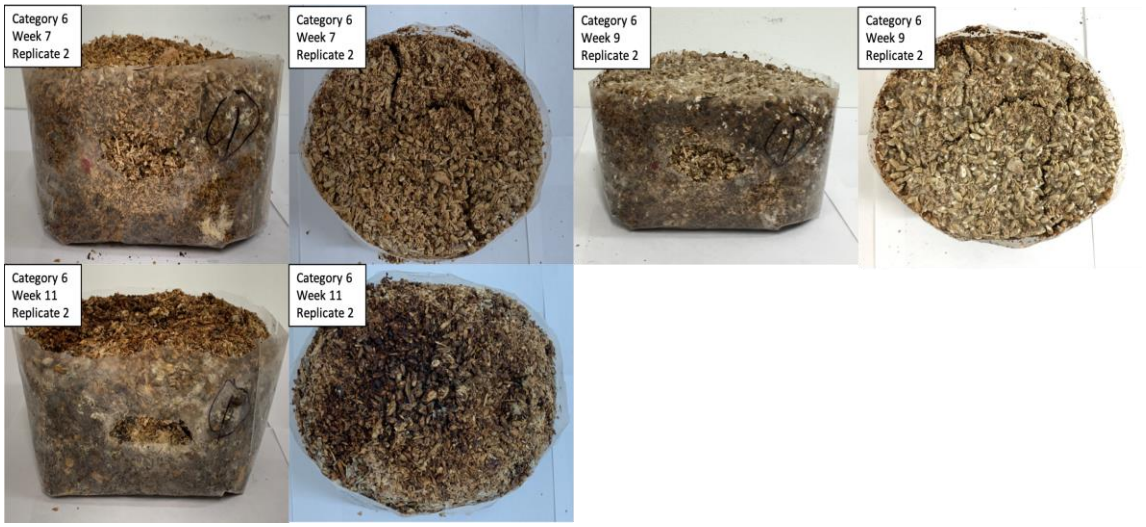


Figure IV-41. Growth bags of replicate 2 of category 6 after cutting top for fruiting body development

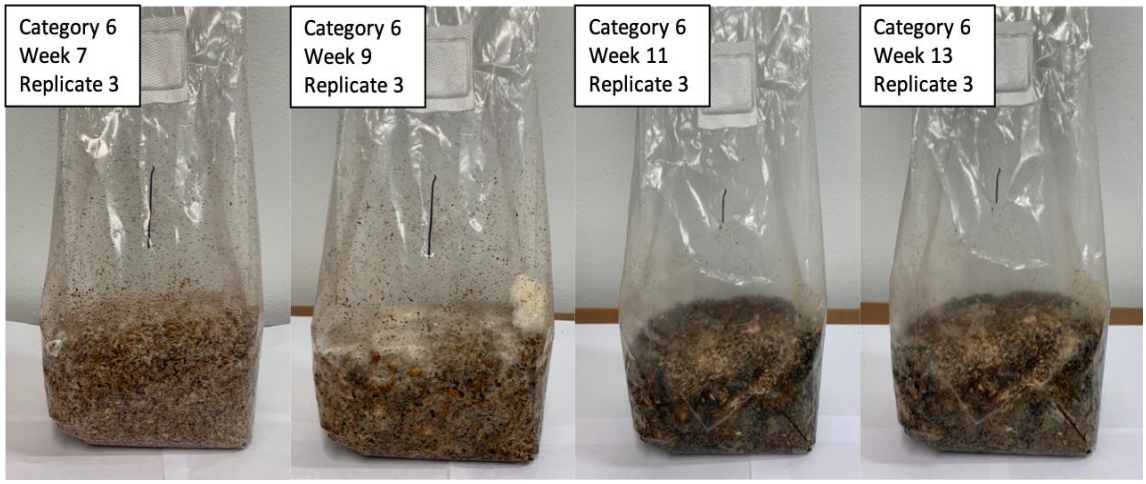
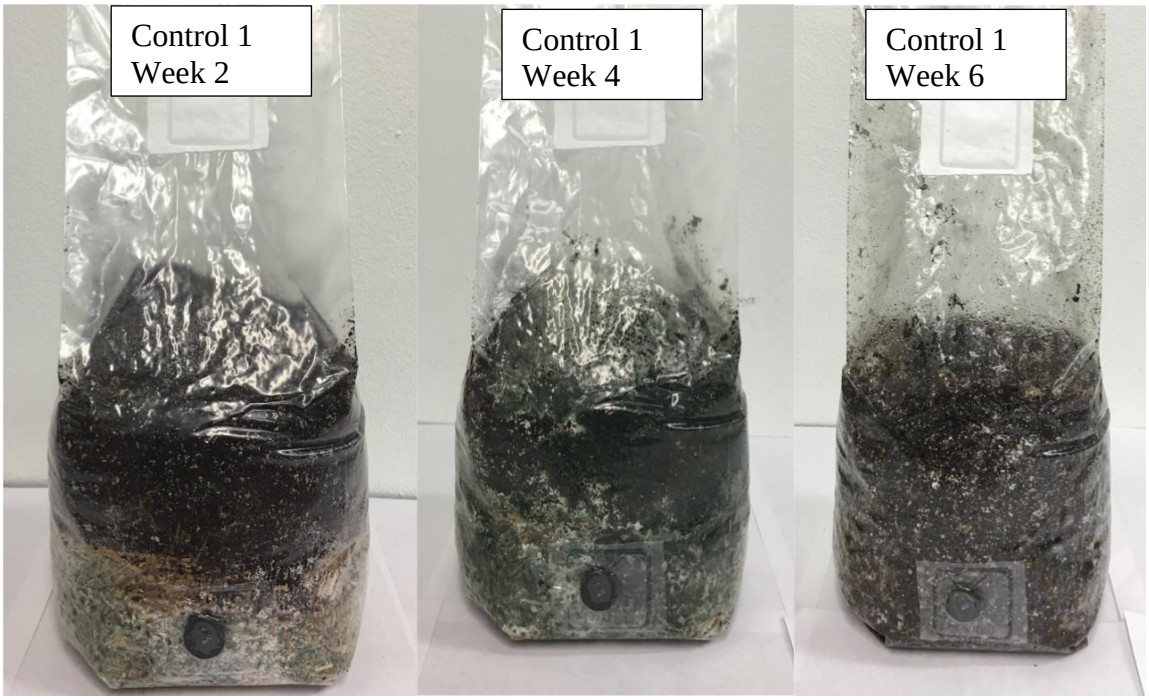


Figure IV-42. Growth bags of replicate 3 of category 6 after X cuts for fruiting body development

IV.5.3 Growth process of control group 1

In Figure IV-43, Figure (a) showed growth bags of control group 1 after two weeks from spore injection. After 4 weeks from spore injection, mycelium grew and increased, Figure (b). Later, the top layer and bottom layer were mixed for further colonization, Figure (c).



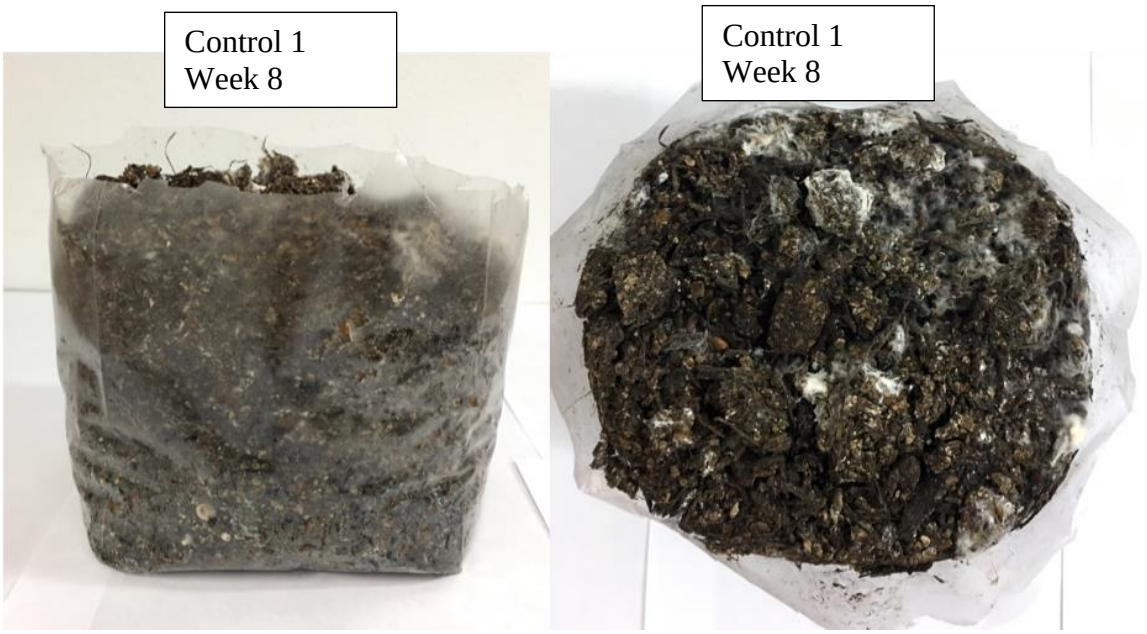
(a) Two weeks after inoculation

(b) Four weeks after inoculation

(c) Six weeks after inoculation and substrate was mixed immediately

Figure IV-43. Growth process of first type of control group

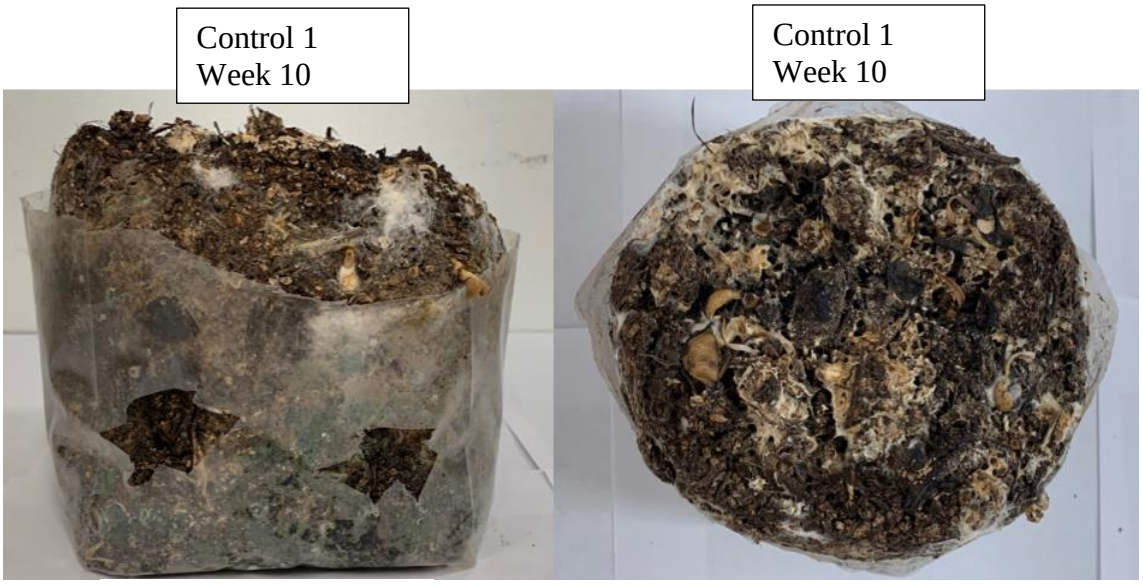
After 2 more weeks of further mycelium colonization, it was time for fruiting body development. The top of the bag was cut off for air exchange and watering, Figure IV-44.



(a) Front view **(b) Top view**

Figure IV-44. Control group 1 - 8 weeks after inoculation and 2 weeks after substrate mixing then cut the top immediately

After another two weeks, there were still some mycelium and no fruiting body of wood ear mushroom appeared. However, some other fungus appeared in growth bags. It may invade into growth chamber through air pathway.



(a) Front view **(b) Top view**

Figure IV-45. Control group 1 - Ten weeks after inoculation and four weeks after substrate mixing

IV.5.4 Growth process of control group 2

Mycelium appeared after two weeks of spore inoculation, Figure (a). After four weeks of mycelium growth, more white mycelium appeared, Figure (b). After six weeks of mycelium growth, it was ready to mix the substrates in the bags. And the substrates were mixed in the bags immediately, Figure (c).

(a) Two weeks after inoculation

(b) Four weeks after inoculation

(c) Six weeks after inoculation and substrate was mixed immediately

Figure IV-46. Growth process of second type of control group

For another two weeks mycelium growth after substrates mixing, it was ready for the fruiting body development stage. Some X cuts were made to let the mycelium get fresh air and water.

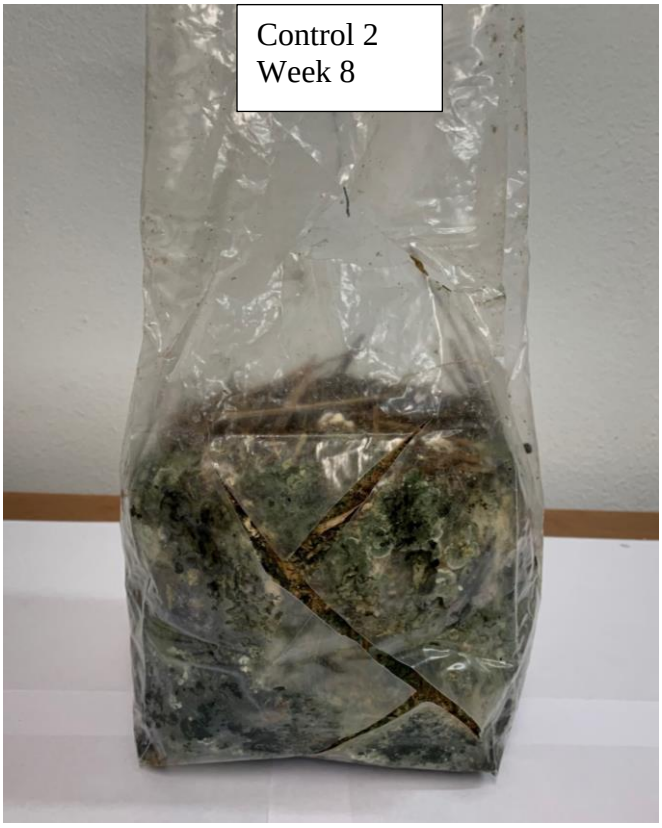


Figure IV-47. Control group 2 -Eight weeks after inoculation and two weeks after substrate mixing then made some X cuts on bag

After two weeks for fruiting body growth, it showed some black fungus in the bags, Figure IV-48. But it died instantly. Some mycelium was still left, but mycelium was decreasing during this period.

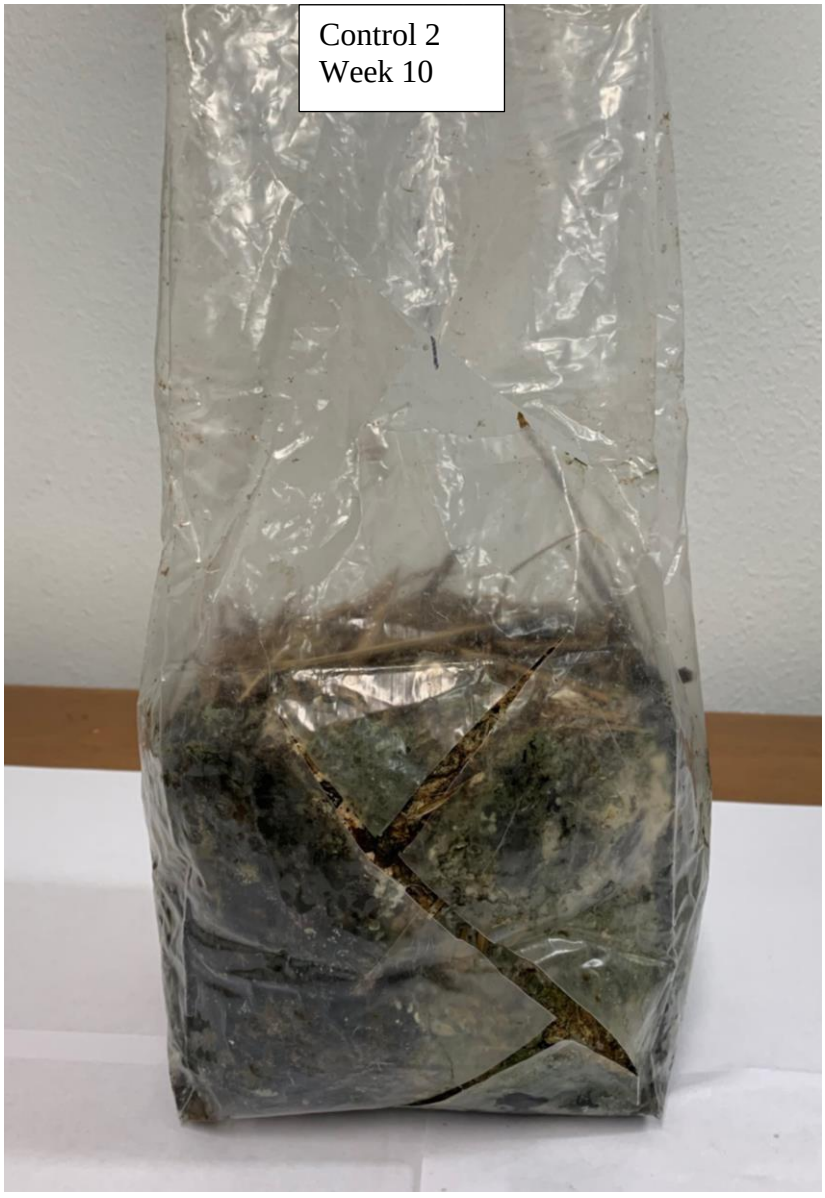


Figure IV-48. Control group 2 - Ten weeks after inoculation and four weeks after substrate mixing

IV.6 Results and Discussion

IV.6.1 The temperature and relative humidity inside growth bags

Hobo data logger sensors were put inside growth bags during the fruiting period after the bags were cut. Figure IV-49 to Figure IV-52 showed the temperature and relative humidity in growth bags of both side of growth chamber.

The numbers on the horizontal axis represented the time and the measurement interval of the data logger was 5 min. The vertical axis showed the temperature inside the growth bags. From Figure IV-49, the temperature varied from 22 to 25 ℃, which was suitable for fruiting body growth.

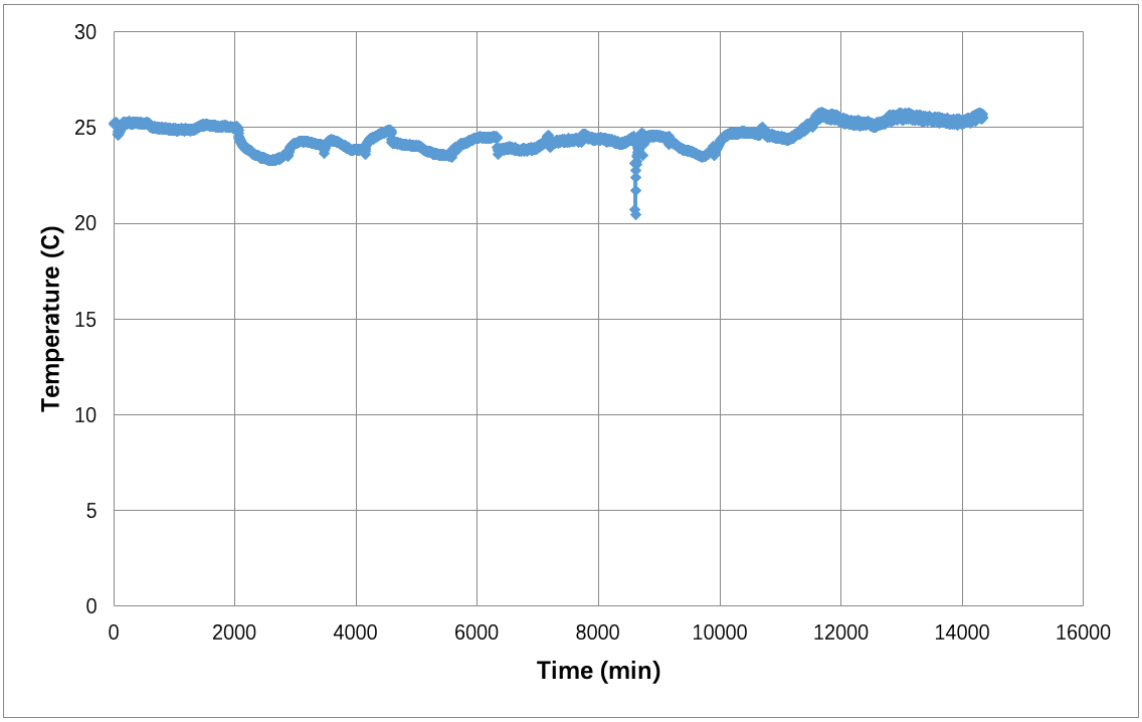


Figure IV-49. Temperature inside growth bags of left side of growth chamber

For Figure IV-50, the vertical axis represented relative humidity. From the figure, the relative humidity varied from 65 to 98 %. For almost 50 % of the total growth time, relative humidity was above 80 % which was optimal for fruiting body development.

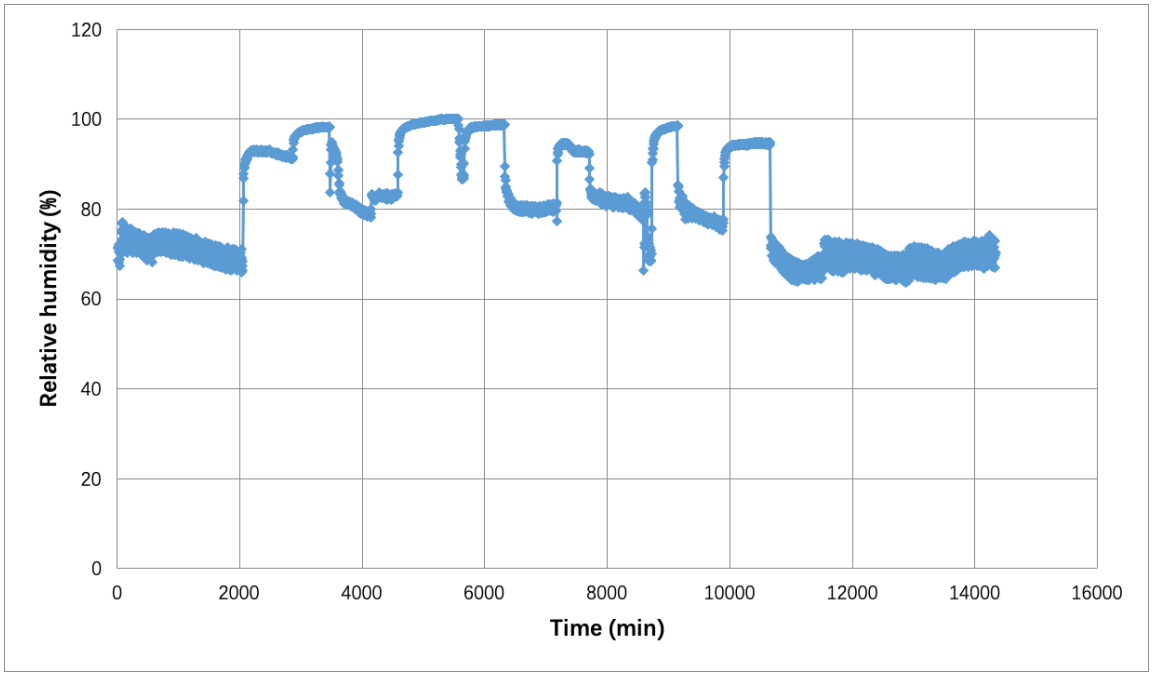


Figure IV-50. Relative humidity inside growth bags inside left side of growth chamber

From Figure IV-51, it also showed the temperature varied from 23.5 to 25.8 ℃, which was optimal for fruiting body growth.

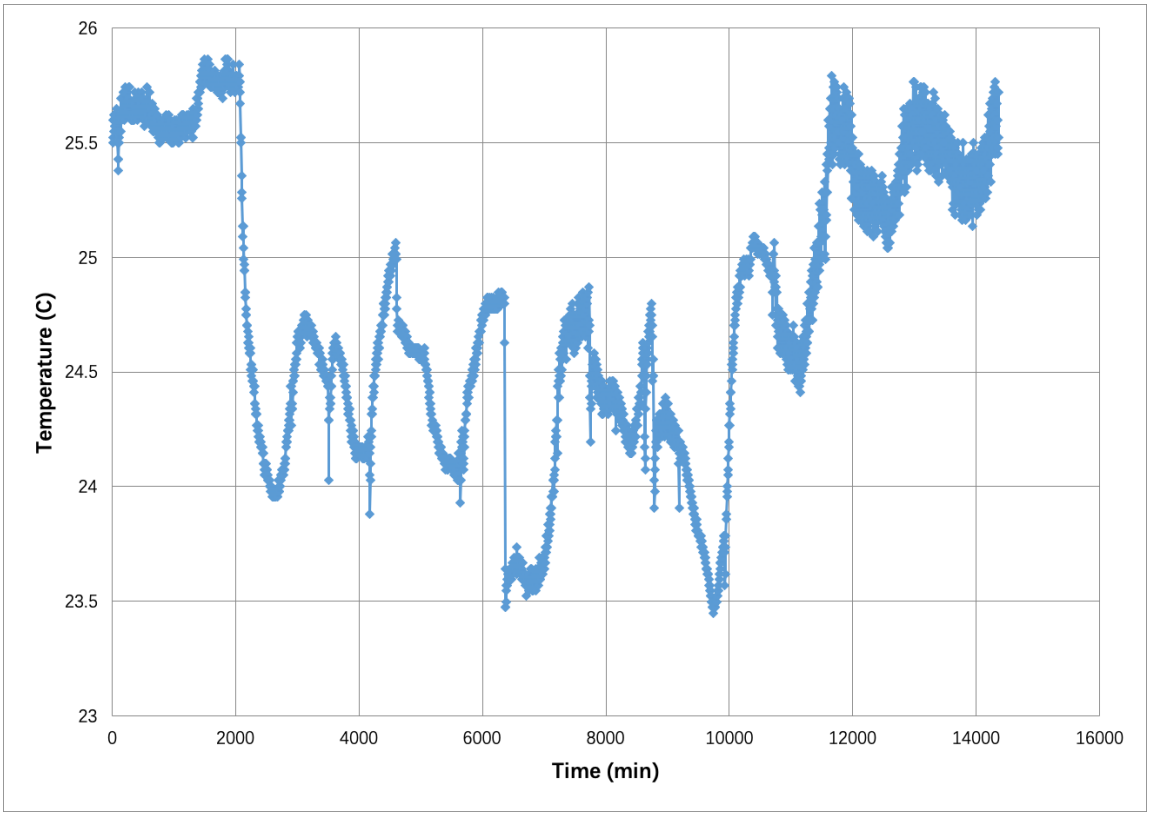


Figure IV-51. The temperature inside bags of right side of chamber

The relative humidity varied from 65 to 98 % inside growth bags in the right side of growth chamber. Most of the time, the relative humidity was higher than 80 %, which can provide enough humidity for fruiting body development.

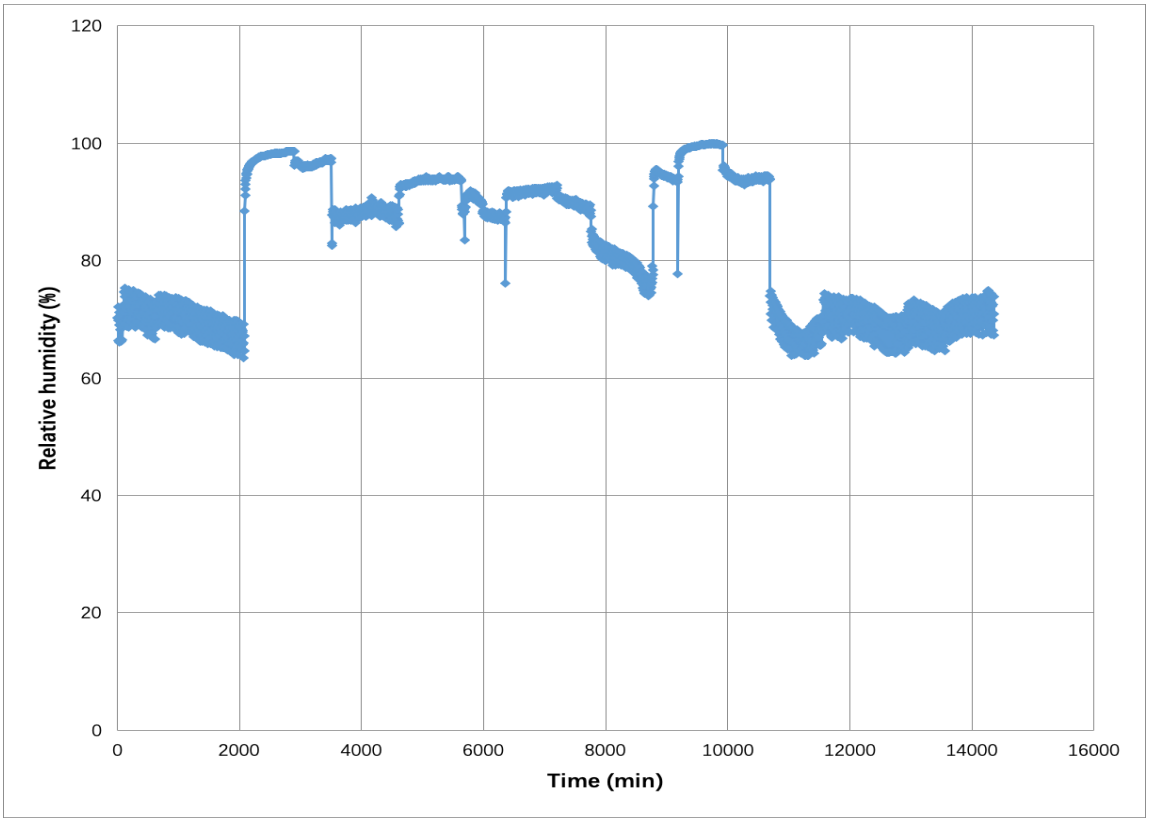


Figure IV-52. Relative humidity inside bags of right side of chamber

IV.6.2 The chemical properties of the wood waste for bag cultivation

The following table and figure showed the carbon, nitrogen contents and C/N ratio of the logs ground to chips as substrate for bag cultivation.

Table IV-2. C/N ratio of wood waste substrate

Wood waste substrates based on density categories ($kg \cdot m^{-3}$)	Average densities of wood substrate at each density category ($kg \cdot m^{-3}$)	Carbon content (%)	Nitrogen content (%)	C/N ratio
700 – 800 (6)	720.97	41.119	0.297	44.12
600 – 700 (5)	648.74	34.856	0.461	22.87
500 – 600 (4)	551.51	43.383	0.32	87.60
400 – 500 (3)	420.47	42.687	0.487	135.62
300 – 400 (2)	385.36	16.108	0.704	75.65
200 – 300 (1)	284.68	27.753	0.629	138.39

Carbon content decreased with increasing density, Figure IV-53. However, P-value was 0.15 which was larger than 0.05, Table IV- 3. Hence, the relation was not significant. It indicated that carbon content of wood waste increased sligtly during decay process.

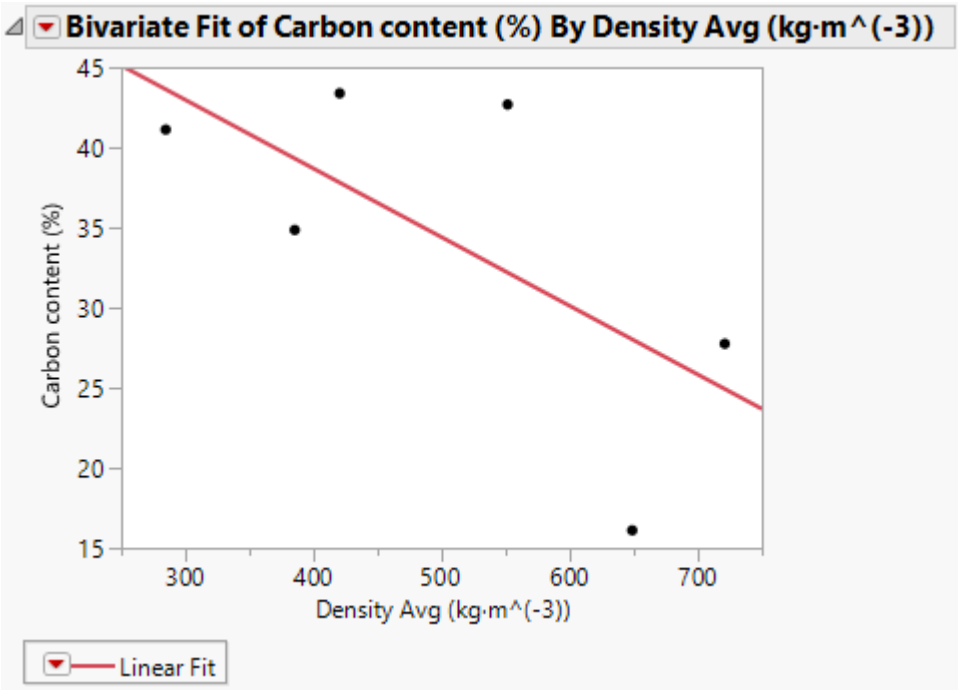


Figure IV-53. Densities versus carbon contents of wood waste substrate

Table IV-3. The ANOVA analysis of densities versus carbon contents of wood waste substrates

Summary of fit	RSquare		0.45
	Rsquare Adj		0.31
	Root Mean Square Error		8.91
	Mean of Response		34.32
	Observations (or Sum Wgts)		6
Analysis of Variance	F Ratio		3.23
	Prob > F		0.15
Parameter Estimates	Intercepts	Estimate	55.83
		t Ratio	4.46
		Prob > |t|	0.0111
	Density Avg	Estimate	-0.04
		t Ratio	-1.80
		Prob > |t|	0.15

For nitrogen content versus density, the positive relationship existed, Figure IV-54. And the P-value was 0.02 that less than 0.05, Table IV- 4. Therefore, the relationship between density and nitrogen content was significant. Hence, the nitrogen content decreased significantly during decomposition.

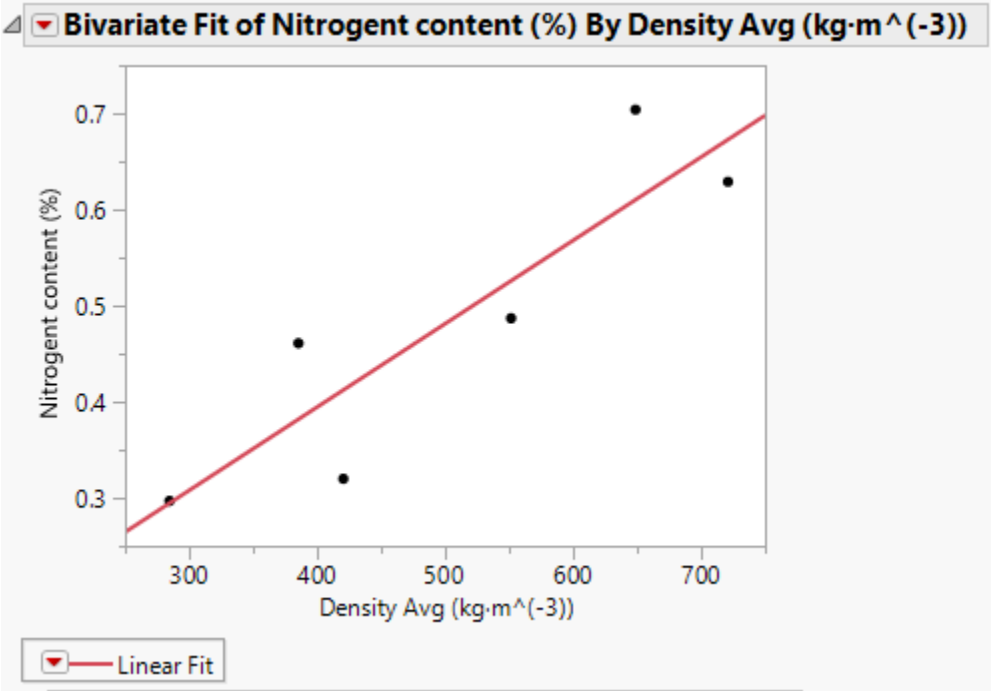


Figure IV-54. Nitrogen contents versus average densities of wood waste substrates

Table IV-4. The ANOVA analysis of densities versus nitrogen contents of wood waste substrates

Summary of fit	RSquare		0.80
	Rsquare Adj		0.74
	Root Mean Square Error		0.08
	Mean of Response		0.48
	Observations (or Sum Wgts)		6
Analysis of Variance	F Ratio		15.51
	Prob > F		0.02
Parameter Estimates	Intercepts	Estimate	0.048
		t Ratio	0.41
		Prob > |t|	0.7002
	Density Avg	Estimate	0.0009
		t Ratio	3.94
		Prob > |t|	0.02

For density versus C/N ratio, the negative relationship was also existed, Figure IV-55. The P-value was 0.04 which was less than 0.05, Table - 5. The relationship was significant. So the C/N ratio of wood waste increased during decay process.

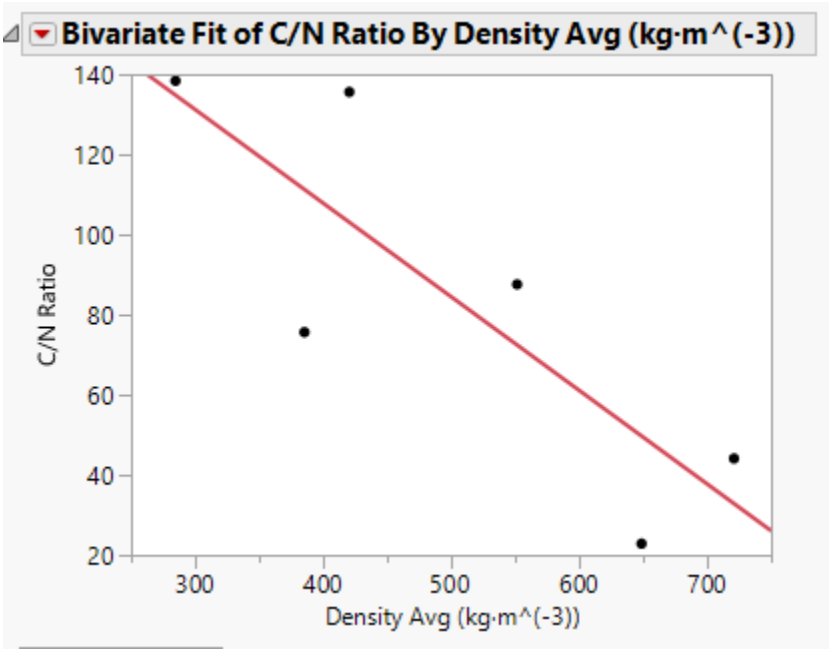


Figure IV-55. The C/N ratio versus densities of wood waste substrate

Table IV-5. ANOVA analysis of density versus C/N ratio of 13 logs with mushroom residue

Summary of fit	RSquare		0.69
	Rsquare Adj		0.61
	Root Mean Square Error		29.24
	Mean of Response		84.04
	Observations (or Sum Wgts)		6
Analysis of Variance	F Ratio		8.91
	Prob > F		0.04
Parameter Estimates	Intercepts	Estimate	201.34
		t Ratio	4.90
		Prob > |t|	0.008
	Density Avg	Estimate	-0.23
		t Ratio	-2.98
		Prob > |t|	0.04

IV.6.3 Mycelium and fruiting body growth

From the bag cultivation process part, it showed that mycelium appeared inside the bags. However, there was no fruiting body in the bags later. The reason may be the shortage of nutrition and mycelium died due to the shortage.

For the fruiting body development stage, all log waste substrates were mixed with the mycelium and rye berry in growth bags, Figure IV- 8. After two more weeks' colonization of mycelium, the top of the bags was cut off for fruiting body development, Figure IV- 9. Although the fruiting body didn't appear, the mycelium growth conditions were different for all bags. The density categories for all bags of 3 replicates from highest to lowest mycelium growth when mycelium growth reached their peak period (two weeks after fruiting body development stage started) were similar, and usually category 1, 2 and 3 had the best growth of mycelium, Figure IV- 10. And the mycelium of bag with wood waste substrate at density category 6 was the lowest growth level.

IV.7 Conclusion

IV.7.1 The best decomposition level for mushroom growth in the bag cultivation method

The bag cultivation method is easier to observe and control than log cultivation. For the growth period from spawn incubation to mycelium growth, all the bags grew successfully. But after adding the wood waste substrate for fruiting, the mycelium didn't show any significant signs of growth. After one and half months, the fruiting body didn't appear. The reason why mycelium didn't result in a fruiting-body may still be the nutrition problem by the wood substrate.

Compared to the experiment group, the bags of the control group showed some signs of fruiting body of other fungus. These fungi may have invaded through the air pathway. However, experiment group didn't get infected by other fungus. The reason

why no fruiting body appeared might still be the nutrition shortage for fruiting body growth.

IV.7.2 The relationship of chemical properties and decay level of wood substrate

The negative relationship between carbon content and decay level of wood waste was existed. This was the same as the previous 18 logs and 13 logs.

For nitrogen content, it increased with increasing density and this relationship was significant. It indicated nitrogen content decreased during the decay process which was also the same compared to 18 and 13 logs.

The same trend existed between C/N ratio and density. C/N ratio increased during decomposition process. Therefore, the same relationship existed between chemical properties and decay level of the 18 logs for mushroom growth, 13 logs with mushroom residue and wood substrate.

CHAPTER V

SUMMARY AND EXPECTATION

V.1 The relationship between wood properties and decay levels of Pecan log

V.1.1 The correlations between wood physical properties and decomposition levels of Pecan logs

From previous chapter, the decay levels of all Pecan logs were determined by the traditional visual methods. It is concluded that fresh logs usually have high densities and moisture contents. And, that old decayed logs always have lower densities and moisture contents. Therefore, the densities can be used as a basic standard to represent the wood decay levels.

The hardness of logs was regarded as an important physical property for the logs. It was measured as penetration depth in this research. From the previous part, there was no significant relationship between log densities and penetration depths when the log densities were larger than 430 $kg \cdot m^{-3}$. However, for Pecan logs, the penetration depths significantly increased with decreasing log densities when the log densities were below 430 $kg \cdot m^{-3}$. Hence, it could be concluded that the penetration depths increased during the log decomposition process.

V.1.2 Chemical properties variations during log decomposition process

As the enzymes activities decomposed logs, the carbon and nitrogen contents were changed. The chemical properties of Pecan logs at different decay levels were

measured. However, the variation in the carbon contents of the logs in this study did not show a significant linear relationship with log densities. There was only a slight trend shown where carbon contents increased slightly with decreasing log densities. Hence, there was slight evidence that the carbon contents increased during pecan log decay process within the range of log densities tested in this study.

For the nitrogen contents, there was no significant linear relationship with pecan log densities. But there was a slight trend where the nitrogen contents decreased when log densities decreased. Hence, nitrogen contents might decrease slightly during the log decomposition process within the range of log densities tested in this study.

However, discussing the carbon or nitrogen contents separately was not as important as the C/N ratio. For all the logs, the linear relationship between C/N ratio and pecan log densities was not significant. There was a slight trend where the C/N ratio increased with decreasing log densities but there was wide variation in the data.

Previous research has shown that during the log decay process, the carbon contents increased significantly with decreasing densities. And nitrogen contents also increased significantly when densities decreased. However, the changing of nitrogen contents was more significant then carbon contents. Hence, the C/N ratio decreased significantly with decreasing densities. It concluded that C/N ratio decreased during log decomposition process. The boreal hardwood trees were observed in Canada in previous research compared to Pecan tree in Texas in this research.

V.2 The preferred decay levels of Pecan logs for Auricularia mushroom growth by log cultivation method

For log cultivation of wood ear mushroom, the fruiting body did not appear. However, some white mycelium appeared in some logs within Category 1, 2 3 and 4 (density range from 200 to 580 $kg \cdot m^{-3}$). It could indicate that logs at low density categories had more mycelium growth compared to logs at high density categories.

For the 6 logs out of the 13 logs with mushroom fruiting residue, that were put inside the growth chamber, only one log grew some new wood ear mushroom fruiting bodies. This log had a density of 502 $kg \cdot m^{-3}$ at Category 4 and C/N ratio of 74.75. For all the logs with known mushroom residue, the average density was 517.85 $kg \cdot m^{-3}$, and standard deviation of density was 60.55. The average value of C/N ratio was 59.15 and standard deviation was 11.52. Hence, this would suggest that log density of around 400 – 550 $kg \cdot m^{-3}$ at category 3 to 4 and C/N ratio at 60 – 80 were preferred for wood ear mushroom growth.

V.3 The optimal decomposition level of wood substrates for wood ear mushroom growth by bag cultivation method

By grew mushroom in growth bags, this cultivation method is easier to observe and control than log cultivation. For the growth period from spawn incubation to mycelium growth, all the bags grew successfully. But after adding the wood waste substrate for fruiting, the mycelium did not show any significant signs of growth. However, the mycelium growth conditions were different for all bags. Density at

category 1, 2 and 3 had better mycelium growth than other density categories. After one and half months, the fruiting body did not appear. The reason why mycelium did not result in a fruiting-body may still be the nutrition problem by the wood substrate.

Compared to the experiment group, the bags of the control group showed some signs of fruiting body of other fungus. These fungi may have invaded through the air pathway. However, experiment group did not get infected by other fungus. The reason why no fruiting body appeared might still be the nutrition shortage for fruiting body growth.

V.4 Expectation and hypothesis for mushroom growth in future

Based on log and bag cultivation method of wood ear mushroom growth, even no mature mushroom fruiting body were harvested, but still got some directions for mushroom growth. Log cultivation and bag cultivation method are both used by many people. But bag cultivation is easy to control environment conditions and grow mushrooms in massive production.

From the research, neither the new or old logs are suitable for wood ear mushroom growth. For what decay level and C/N ratio of logs are best for mushroom growth, it still needs further exploration. While during the mushroom growth life cycle, the spawn incubation to mycelium growth were usually easy and successful. The key is the fruiting body appearance and development. If the nutrition condition is not satisfied, the growth stage would be inhibited and stop.

For future research on mushroom growth, I would recommend growing mushrooms in a big green house rather than a growth chamber. To provide a better air exchange environment, big space is much better. Stagnant air can cause fruiting body deformity and appearance of toadstools. Also, big green houses can use big and efficient humidifiers to guarantee the high air humidity. The requirement for sterile environment is not really strict, but still need to care about the infection of other fungus. The completed sterilization work needs to be done before growth, and always check the environment to prevent fungus infection. The watering during fruiting body period also needs to be done carefully, watering too much or too little can both inhibit mycelium growth and fruiting body appearance. Even mushroom growth doesn't need high light intensity, but light is important to induce fruiting body growth. Follow strictly to the light requirement during mushroom growth.

REFFERENCE

Angers, V. A., Drapeau, P., & Bergeron, Y. (2011, Dec). Mineralization rates and factors influencing snag decay in four North American boreal tree species. Canadian Journal of Forest Research, 42(1), 157-166.

Angers, V. A., Pierre, D., & Bergeron, Y. (2010). Snag degradation pathways of four North American boreal tree species. Forest Ecology and Management, 259, 246-256.

Berry, F., & Beaton, J. (1972, January). Decay in oak in the Central Hardwood Region. USDA forest research paper, 242.

Brais, S., Quideau, S., Kebli, H., & Drapeau, P. (2013, September). Chemical transformations in downed logs and snags of mixed boreal species during decompostion. Canadian Journal of Froest Research, 43, 785-798.

Creed, I., Webster, K., & Morrison, D. (2004, March). A comparison of techniques for measuring density and concentrations of carbon and nigrogen in coarse woody debris at different stages of decay. NRC Canada, 34, 744-753.

Department of Forest Science. (1999). Relationship between relative wood density of Picea abies logs and simple classification systems of decayed coarse woody debris. Scandinavian Journal of Forest Research, 14(5), 454-461.

Department of Forest Sciences. (2010, Nov). Relationshiop between relative wood density of Picea abies logs and simple classification systems of decayed coarse woody debris. Scandinavian Journal of Forest Research, 14(4), 454-461.

Greaves, B., Raymond, C., Borralho, N., & Farrington, A. (1996). Use of a Pilodyn for the indirect selection of basic density in Eucalyptus nitens. Canadian Journal of Forest Research, 26(9), 1643-1650.

Hale, C. M., & Pastor, J. (1998, September). Nitrogen content, decay rates, and decompositional dynamics of hollow versus solid hardwood logs in hardwood forests of Minnesota, U.S.A. Canadian Journal of Forest Research, 28(9), 1276-1285.

Jonason, S., & Fasidi, I. (2001). Effect of carbon, nitrogen and mineral sources on growth of Psathyerella atroumbonata (Pegler), a Nigerian edible mushroom. Food Chemistry, 72, 479-483.

Kadnikova, I. A., Costa, R., Kalenik, T. K., Guruleva, O. N., & Shi, Y. (2015, Oct). Chemical Composition and Nutritional Value of the Mushroom Auricularia auricula-judae. Journal of Food and Nutrition Research, 3(8), 478-482.

Katsuji, Y. (1997). Production of cultivated edible mushrooms. Food Reviews International, 13(3), 327-333.

Kruys, N., Jonsson, B. G., & Stahl, G. (2002). A stage-based matrix model for decay-class dynamics of woody debris. Ecological Society of America, 12(3), 773-781.

Larjavaara, M., & Muller-Landau, H. C. (2010, December). Comaparison of decay classification, knife test, and two penetrometers for estimating wood density of coarse woody debris. Canadian Journal of Forest Research, 40(12), 2313-2321.

Larjavaara, M., & Muller-Landau, H. C. (2010, December). Comparison of decay classfication, knife test, and two penetrometers for estimating wood density of coarse debris. Canadian Journal of Forest Research, 40(12), 2312-2321.

Luo, X. (1993). Biology of Artifical Log Cultivation of Auicularia Mushrooms. Hong Kong, China: Chinese University Press.

Luo, Y., Chen, G., Li, B., Ji, B., Guo, Y., & Tian, F. (2008). Evaluation of antioxidative and hypolipidemic properties of a novel functional diet formulation of Auricularia auricula and Hawthorn. Innovative Food Science and Emerging Technologies, 10, 215-221.

Makippa, R., & Linkosalo, T. (2011). A Non-Destructive Field Method for Measuring Wood Density of Decaying Logs. Silva Fennica, 45(5), 1135-1142.

Melillo, J. M., Aber, H. D., Linkins, A. E., Ricca, A., Fry, B., & Nadelhoffer, K. J. (1989, April). Carbon and nigrogen dynamics along the decay continuum: plant litter to soil organic matter. Plant and Soil, 115(2), 189-198.

Rajala, T., Peltoniemi, M., Pennanen, T., & Makipaa, R. (2010, Nov). Relationship between wood-inhabiting fungi determined by molecular analysis (denaturing gradient gel electrophoresis) and quality of decaying logs. Canadian Journal of Forest Research, 40(12), 2384-2397.

Rayner, A., & Boddy, L. (1988). Fungal decomposition of wood. Its biology and ecology. Bath, Avon, UK: John Wiley & Sons Ltd.

Shigo, A. L. (1965, June). The pattern of decays and discolorations in northern hardwoods. Phytopathology, 55, 648-652.

Zhang, B., & Chen, J. (2011, Sep.). Determination and Analysis of Nutrition Components in Sarcodon aspratus. Food Science.

Zhang, H., Wang, Z., Zhang, Z., & Wang, X. (2010, Dec). Purified Auricularia auricular-judae polysaccharide (AAP I-a) prevents oxidative stress in an ageing mouse model. Carbohydrate Polymers, 84(2011), 638-648.

Out-grow.com, Inc. 11321 North Hulbert Road, Mcconnell, IL 61050, (815) 868-1000